PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 June 2001 (12.06.01)	
International application No. PCT/US00/22725	**Applicant's or agent's file reference** 7024473P118
International filing date (day/month/year) 18 August 2000 (18.08.00)	**Priority date** (day/month/year) 20 August 1999 (20.08.99)
Applicant OGAS, Joseph, P. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 March 2001 (19.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

SCHWARTZ, Jason, J.
Woodard, Emhardt, Naughton,
Moriarty & McNett
Bank One Center/Tower
Suite 3700
111 Monument Circle
Indianapolis, IN 46204
ETATS-UNIS D'AMERIQUE

RECEIVED
NOV 20 2000

Date of mailing (day/month/year) 02 November 2000 (02.11.00)	
Applicant's or agent's file reference 7024473P118	**IMPORTANT NOTIFICATION**
International application No. PCT/US00/22725	International filing date (day/month/year) 18 August 2000 (18.08.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 20 August 1999 (20.08.99)
Applicant PURDUE RESEARCH FOUNDATION et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
20 Augu 1999 (20.08.99)	60/149,975	US	26 Octo 2000 (26.10.00)

The Internati nal Bureau of WIPO
34, chemin des Col mbett s
1211 G n va 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Tessadel PAMPLIEGA

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
SCHWARTZ, Jason, J.
Woodard, Emhardt, Naughton,
Moriarty & McNett
Bank One Center/Tower
Suite 3700
111 Monument Circle
Indianapolis, IN 46204
ETATS-UNIS D'AMERIQUE

RECEIVED
MAR 1 6 2001
Woodard, Emhardt, Naughton, Moriarty & McNett

Date of mailing (day/month/year) 01 March 2001 (01.03.01)	
Applicant's or agent's file reference 7024473P118	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US00/22725	18 August 2000 (18.08.00)	20 August 1999 (20.08.99)

Applicant
PURDUE RESEARCH FOUNDATION et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AG,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EA,EE,EP,ES,
   FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,
   MN,MW,MX,MZ,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   01 March 2001 (01.03.01) under No. WO 01/14519

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

Th International Bureau of WIPO
34, ch min des Col mbett s
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

ATENT COOPERATION TRE TY

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

SCHWARTZ, Jason, J.
Woodard, Emhardt, Naughton,
Moriarty & McNett
Bank One Center/Tower
Suite 3700
111 Monument Circle
Indianapolis, IN 46204
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)	
12 June 2001 (12.06.01)	
Applicant's or agent's file reference 7024473P118	**IMPORTANT INFORMATION**

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US00/22725	18 August 2000 (18.08.00)	20 August 1999 (20.08.99)

Applicant
PURDUE RESEARCH FOUNDATION et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   AP :GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW
   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AE,AG,AL,AM,AT,AZ,BA,BB,BR,BY,BZ,CH,CR,CU,DK,DM,DZ,EE,ES,FI,GB,
   GD,GE,GH,GM,HR,HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MW,
   MX,MZ,PT,SD,SG,SI,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

Claudio Borton

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

RECEIVED

APR 0 8 2001

Woodard, Emhardt, Naughton, Moriarty & McNett

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

JASON J. SCHWARTZ
WOODARD, EMHARDT, NAUGHTON, MORIARTY &
MCNETT; BANK ONE CENTER/TOWER
SUITE 3700, 111 MONUMENT CIRCLE
INDIANAPOLIS IN 46204

PCT

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	06 APR 2001
Applicant's or agent's file reference: 7024473P118	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/22725	18 AUG 00	20 AUG 99

Applicant: PURDUE RESEARCH FOUNDATION

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

19 March 2001 (19.03.01)

2. That date of receipt is:

☑ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION**: That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/US Assistant Commissioner for Patents Box PCT Washington, D.C. 20231 Attn: IPEA/US Facsimile No.	Authorized officer: Jeffrey M. Johnson-Vessels Supervisory Paralegal Specialist Team 1 PCT Operations - IAPD Telephone No. (703) 305-3624 (703) 305-3230(FAX)

Form PCT/IPEA/402 (July 1998)

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: SCHWARTZ, JASON J.
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
BANK ONE CENTER/TOWER, SUITE 3700
111 MONUMENT CIRCLE
INDIANAPOLIS, INDIANA 46204

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	29 JUN 2001
Applicant's or agent's file reference: 7024473P118	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/22725	18 AUGUST 2000	20 AUGUST 1999

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

ENTERED 8-29-01

Applicant
PURDUE RESEARCH FOUNDATION

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [X] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [X] Certain defects in the international application
- VIII [X] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20 DECEMBER 2001.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

ASHWIN MEHTA

Form PCT/IPEA/408 (cover sheet) (July 1998) *

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: SCHWARTZ, JASON J.
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
BANK ONE CENTER/TOWER, SUITE 3700
111 MONUMENT CIRCLE
INDIANAPOLIS, INDIANA 46204

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	29 JUN 2001
Applicant's or agent's file reference: 7024473P118	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/22725	18 AUGUST 2000	20 AUGUST 1999

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

Applicant
PURDUE RESEARCH FOUNDATION

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [X] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [X] Certain defects in the international application
- VIII [X] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20 DECEMBER 2001.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600 ASHWIN MEHTA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (July 1998) ★

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees the applicant has:

- ☐ restricted the claims. (See Supplemental Sheet)
- ☒ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1 not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

- ☒ all parts.
- ☐ the parts relating to claims Nos. _.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. citations and explanations

Claims 1, 4-10, 20-23, 26-27, 30, 33, 35, 37, 55, 58-60, 63-65, 67, 70, 71, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Jin et al.

Jin et al teach the isolation and characterization of the hrp1+ gene. It contains a chromodomain, a helicase domain, and a DNA-binding domain, and acts as a negative regulator of cell growth. Jin et al also teach overexpression of hrp1+ in yeast cells (pages 321-324, 326).

Claims 55-60, 63-65, 67-69, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Woodage et al.

Woodage et al teach the characterization of CHD family of proteins. Numerous proteins having chromo domains, helicase domains, and DNA binding domains are taught, for example the murine CHD1 protein, and the gene which encodes it (page11472-11474). CHD proteins which further contain a zinc-finger domain and/or a second chromo domain are also taught, for example HsCHD3 (pages 11473 and 11475). Yeast strains transformed with ScCHD1 are also taught (page 11473).

Claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, and 83 meet the criteria set out in PCT Article 33(2) and (3), because the prior art does not teach or fairly suggest SEQ ID NO: 1, the Arabidopsis PKL gene or protein, a method of transforming a host cell with any of the claimed nulceotide sequences which include sequences encoding a zinc finger or a second chromo domain, or wherein the host cell is an animal or plant cell. Claims 1-82 meet the criteria set out in PCT Article 33(4) in that the claimed nucleotide sequences may be used to control the development of agriculturally important organisms.

---------- NEW CITATIONS ----------

NONE

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claims 2 and 43 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: The claims are exactly identical.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 4-9, 20, 26, 27, 33-35,37,40-42, 45-47, 49-52, 54, 58-61, 63-65, 70, 71, 73-78, 80, 81, and 83 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s) : The recitations "at least about" and "about" render the claims indefinite. The boundaries of the indicates nucleotide sequences are not made clear by the recitations, and therefore the metes and bounds of the claims are unknown.

Claims 16 and 17 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): The recitation "lysinse 304" in claim 16 renders the claims indefinite. The recitation is apparently making reference to a particular amino acid sequence. However, the identity of this sequence is not known.

Claims 28, 60, 62, 71, 72, 79-83 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): The claims refer to the amino acid sequence of SEQ ID NO: 1. However, SEQ ID NO: 1 is a nulceotide sequence.

Claims 55-57 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): It is not clear what the claims are drawn to. The recitation "identity.SEQ ID NO: 1;" in line 6 of claim 55 does not make sense. Further, dependent claims 56 and 57 refer to the "method of claim 55".

Claims 18, 24, 47, 78 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the term "PKL" renders the claims indefinite. The description at page 10, lines 24-26 indicates that all of the described proteins are generally referred to as "PKL". It is therefore not clear which particular PKL protein the claims are referring to.

Claim 76 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): the reciation "said nucleotide sequence in lines 1-2 and in line 3 renders the claim indefinite. The claim seems to indicate that the nucleotide sequence is complementary to itself.

Claim 24 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): There is no antcedent basis for (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 5/04, 15/00, 15/12, 15/29, 15/63, 15/64, 15/82, 15/85, 15/87 15/90; A01H 5/00; C07H 21/02, 21/04 and US Cl.: 435/69.1, 320.2, 410, 419, 455, 468, 471; 536/23.1, 23.5, 23.6; 800/21, 278, 286, 287, 290, 295, 298

IV. LACK OF UNITY OF INVENTION:

1. This response is made to a telephone Lack of Unity requirement (see telephone memorandum attached hereto or attached to a prior Written Opinion).

V. 1. REASONED STATEMENTS:
The opinion as to Novelty was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The opinion as to Novelty was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The opinion as to Inventive Step was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The opinion as to Inventive Step was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The opinion as to Industrial Applicability was positive (YES) with respect to claims 1-82.
The opinion as to Industrial Applicability was negative (NO) with respect to claims NONE.

VIII. CERTAIN OBSERVATIONS ON THE APPLICATION (Continued):
"PKL" in the claim or parent claim 1.

Claim 25 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): the recitation "substantial similarity" renders the claim indefinite. It is not clear what is meant by this recitation.

The description is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 5 because it fails to adequately enable practice of the claimed invention because: it does not enable the claimed method when applied to non-plant organisms. The description teaches the PKL protein of Arabidopsis, that it is a "CHD" protein, and a method of expressing it in plants (pages 25-49). Ogas et al teach that PKL may function to establish repression of embryonic identity during germination, and is responsive to the presence of gibberellin (page 13839). Ogas et al also teach that it remains to be determined whether other CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events. At the time the application was filed, therefore, it was not known whether animal CHD proteins could be used in methods to regulate development of host cells. It is therefore unpredictable that the claimed method can regulate developmental identity of all host cells. It is clear even to the uninitiated that plant systems are quite different from animal systems, and that a role of PKL in plant germination cannot be extrapolated to a role in animal development. In the absense of further guidance, undue experimentation would be required by one skilled in the art to use the claimed method to regulate the development of all host cell types.

Claims 1-10, 12-30, 32-69 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the immediately preceding paragraph.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 7024473P118	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/22725	International filing date *(day/month/year)* 18 AUGUST 2000	Priority date *(day/month/year)* 20 AUGUST 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant PURDUE RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets.

   [1] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 18 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [X] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [X] Certain defects in the international application

   VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19 MARCH 2001	19 OCTOBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ASHWIN MEHTA Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/22725

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/22725

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [X] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-37, 55-57, and 77-79, drawn to a first method of transforming any host cell, comprising introducing into said cell a nucleic acid molecule encoding a protein having at least one chromo domain, a helicase domain, and a DNA binding domain, or wherein said protein encodes PKL; a first product- a recombinant nucleic acid molecule comprising said nucleic acid molecule and a foreign promoter, and a transgenic plant comprising said nucleic acid molecule.
Group II, claim(s) 38-54, and 76, drawn to a second method, of transforming any host cell, comprising introducing into any host cell an antisense DNA or RNA molecule; and a second product, a transgenic plant comprising said antisense DNA or RNA molecule.
Group III, claim(s) 80-83, drawn to a third product, a recombinant protein, and a third method, of producing a PKL protein.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of Group I does not share the nucleic acid molecule with Group II or III. The antisense molecules of the method of Group II are not shared with the method or nucleotide sequences of Group I, nor the protein and method of Group III. The protein of Group III is not shared with any of the other groups.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. _.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/22725

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. citations and explanations (Rule 70.7)

Claims 1, 4-10, 20-23, 26-27, 30, 33, 35, 37, 55, 58-60, 63-65, 67, 70, 71, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Jin et al.

Jin et al teach the isolation and characterization of the hrp1+ gene. It contains a chromodomain, a helicase domain, and a DNA-binding domain, and acts as a negative regulator of cell growth. Jin et al also teach overexpression of hrp1+ in yeast cells (pages 321-324, 326).

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants concede that hrp1+ may produce a gene involved in gene expression generally and that its function may relate to cell growth, but argue that Jin et al do not teach or suggest hrp1+ being involved in developmental identity. Applicant's arguements have been fully considered but were not found persuasive. As hrp1+ is a yeast gene whose product is involved in regulating cell growth, it can be considered as being involved in the regulation of development of yeast cells. As written, the claimed invention is anticipated by Jin et al.

Claims 55-60, 63-65, 67-69, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Woodage et al.

Woodage et al teach the characterization of CHD family of proteins. Numerous proteins having chromo domains, helicase domains, and DNA binding domains are taught, for example the murine CHD1 protein, and the gene which encodes it (page11472-11474). CHD proteins which further contain a zinc-finger domain and/or a second chromo domain are also taught, for example HsCHD3 (pages 11473 and 11475). Yeast strains transformed with ScCHD1 are also taught (page 11473).

Applicants traverse the rejection in the paper submitted 28 August 2001. Applicants argue that Woodage et al do not teach or suggest a nucleic acid sequence that codes for a protein that has the recited domains and functions to regulate developmental identity. Applicant's arguements have been fully considered but were not found persuasive. That the proteins taught by Woodage et al have function in regulating developmental identity would be property inherent to them. (Continued on Supplemental Sheet.)

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claims 2 and 43 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: The claims are exactly identical.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that the claims are dependent on different independent claims, of different scope. However, both claims 2 and 43 are dependent on claim 1.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 4-9, 20, 26, 27, 33-35,37,40-42, 45-47, 49-52, 54, 58-61, 63-65, 70, 71, 73-78, 80, 81, and 83 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): The recitations "at least about" and "about" render the claims indefinite. The boundaries of the indicates nucleotide sequences are not made clear by the recitations, and therefore the metes and bounds of the claims are unknown.

Claims 56 and 57 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): Dependent claims 56 and 57 refer to the "method of claim 55", which is drawn to a product.

Claims 18, 24, 47, 78 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the term "PKL" renders the claims indefinite. The description at page 10, lines 24-26 indicates that all of the described proteins are generally referred to as "PKL". It is therefore not clear which particular PKL protein the claims are referring to.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that one skilled in the art would clearly understand the term "PKL" in light of the description, citing for example that SEQ ID NO: 2 shows one preferred embodiment of PKL, and that variants of the polypeptide are included as described on page 10, and that a description may also be found on pages 11-13. Applicant's arguements have been fully considered but were not found persuasive. The description does not define how PKL is distinguished from other genes encompassed by claim 1. Further "PKL" appears to be an arbitrary designation. It is not clear how one would identify another PKL if others in the art refer to homologs by another designation.

Claim 24 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): There is no antecedent basis for "PKL" in the claim or parent claim 1.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that amended claim 24 is now dependent on claim 18. However, there is still no antecedent basis for "said plant" in claim 24 or in the claims from which it depends.

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII **Sheet 10**

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 5/04, 15/00, 15/12, 15/29, 15/63, 15/64, 15/82, 15/85, 15/87 15/90; A01H 5/00; C07H 21/02, 21/04 and US Cl.: 435/69.1, 320.2, 410, 419, 455, 468, 471; 536/23.1, 23.5, 23.6; 800/21, 278, 286, 287, 290, 295, 298

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-9, 13-23, 25, 27-30, 32-41, 49, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 10-12, 24, 26, 31, and 42-48, filed with the letter of 28 August 2001.

This report has been drawn on the basis of the claims,
page(s) 50, 54-56, 59, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 51-53, 57, 58, 60, and 61, filed with the letter of 28 August 2001.

This report has been drawn on the basis of the drawings,
page(s) 1-4, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
Pages 1-28, filed wit the letter of 28 August 2001.

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The report as to Novelty was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The report as to Inventive Step was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The report as to Inventive Step was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-82.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, and 83 meet the criteria set out in PCT Article 33(2) and (3), because the prior art does not teach or fairly suggest SEQ ID NO: 1, the Arabidopsis PKL gene or protein, a method of transforming a host cell with any of the claimed nulceotide sequences which include sequences encoding a zinc finger or a second chromo domain, or wherein the host cell is an animal or plant cell. Claims 1-82 meet the criteria set out in PCT Article 33(4) in that the claimed nucleotide sequences may be used to control the development of agriculturally important organisms.

---------- NEW CITATIONS ----------

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

NONE

VIII. CERTAIN OBSERVATIONS ON THE APPLICATION (Continued):

Claim 25 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): the recitation "substantial similarity" renders the claim indefinite. It is not clear what is meant by this recitation.

The description is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 5 because it fails to adequately enable practice of the claimed invention because: it does not enable the claimed method when applied to non-plant organisms. The description teaches the PKL protein of Arabidopsis, that it is a "CHD" protein, and a method of expressing it in plants (pages 25-49). Ogas et al teach that PKL may function to establish repression of embryonic identity during germination, and is responsive to the presence of gibberellin (page 13839). Ogas et al also teach that it remains to be determined whether other CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events. At the time the application was filed, therefore, it was not known whether animal CHD proteins could be used in methods to regulate development of host cells. It is therefore unpredictable that the claimed method can regulate developmental identity of all host cells. It is clear even to the uninitiated that plant systems are quite different from animal systems, and that a role of PKL in plant germination cannot be extrapolated to a role in animal development. In the absense of further guidance, undue experimentation would be required by one skilled in the art to use the claimed method to regulate the development of all host cell types.

Claims 1-10, 12-30, 32-69 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the immediately preceding paragraph.

REGARDING THE INTERNATIONAL APPLICATION OF
PURDUE RESEARCH FOUND. ON, et al.

DOCKET OR REFERENCE NUMBER
24473P118

ENTITLED
METHODS AND COMPOS[redacted]ONS FOR REGULATING DEVELOPMENT[redacted]IDENTITY

Rec'd PCT/PTO 07 FEB 2002

Certification under 37 CFR 1.10 (if applicable)

EL551803433US
"Express Mail" mailing number

18 August 2000
Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Linda S.W. Conrad
(Typed or printed name of person mailing application)

Linda S.W. Conrad
(Signature of person mailing application)

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. [XX] PREPARATION AND TRANSMITTAL OF CERTIFIED COPY OF PRIORITY DOCUMENTS—Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451).

   To cover the cost of copy preparation and certification (37 CFR 1.19(a)(3) and (b)(1)),
   [XX] a (check) (money order) in the amount of $15.00 included in fees is attached to this transmittal letter.
   [ ] the RO/US is hereby authorized to charge the following deposit account no.: _____.

2. [XX] CHOICE OF INTERNATIONAL SEARCHING AUTHORITY—It is requested that the International Search be performed by the following International Searching Authority:

   [XX] United States Patent and Trademark Office (ISA/US)
   [ ] European Patent Office (ISA/EP)

   The appropriate Search fee for the above-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

3. [XX] SUPPLEMENTAL SEARCH FEES (ONLY WHEN ISA/US CONDUCTS THE INTERNATIONAL SEARCH.)—Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account no.: 23-3030.

   I understand that this authorization is subject to my oral confirmation thereof in each instance and that it in no way limits my right to submit a protest against payment of the Supplemental Search fees, but is merely an administrative aid to assure that the ISA/US may timely complete the Search Report

NOTE: SUPPLEMENTAL SEARCH FEES FOR ISA/EP ARE PAYABLE DIRECTLY TO THE EUROPEAN PATENT OFFICE

4. [XX] DISCLOSURE INFORMATION—In order to assist in screening the accompanying International application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied:

   A. [ ] There is no prior filed application relating to this invention.

   B. [XX] There is a prior application, serial number 60/149,975 filed on 20 August 1999 (20.08.99) which contains subject matter that is

      1. [ ] substantially identical to that of the accompanying International application.
      2. [X] less than that of the accompanying International application. The additional subject matter of the International application appears ~~on pages(s) and line(s)~~ throughout application
      3. [ ] more than that of the accompanying International application.

   C. [ ] Disclosure information cannot be covered by the language of Points 4A or 4B above due to the involvement of several prior applications or for other reasons. A separate sheet on which the disclosure information is explained is attached to this transmittal letter.

5. [XX] REQUEST FOR FOREIGN TRANSMITTAL LICENSE—According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International application to foreign agencies or international authorities is hereby requested.

SIGNER IS THE	NAME OF SIGNER (typed)
[ ] APPLICANT [ ] COMMON REPRESENTATIVE [XX] (ATTORNEY) (AGENT) #43,910 REG NO _____	Jason J. SCHWARTZ SIGNATURE

PTO-1382 (REV 3-84)
USCOMM-DC 84-3817

U.S. Department of Commerce
Patent and Trademark Office

This sheet is not p. of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Ann x to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: 7024473P118

Applicant: PURDUE RESEARCH FOUNDATION, et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 240 T
2. SEARCH FEE . . . . . 700 S

International search to be carried out by US

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 91 sheets.

first 30 sheets 427 b1

62 (remaining sheets) x 10 (additional amount) = 620 b2

Add amounts entered at b1 and b2 and enter total at B . . . . 1047 B

Designation Fees

The international application contains 87 designations.

8 (number of designation fees payable *(maximum 8)*) x 92 (amount of designation fee) = 736 D

Add amounts entered at B and D and enter total at I . . . . . 1783 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . 15 P

5. TOTAL FEES PAYABLE . . . . . 2738 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☒ authorization to charge deposit account (see below)	☐ bank draft	☐ coupons
☒ cheque	☐ cash	☐ other *(specify):*
☐ postal money order	☐ revenue stamps	

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ US

☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

23-3030 (Deposit Account No.)

18/08/2000 (Date *(day/month/year)*)

Signature: Jason J. SCHWARTZ, #43,910

See Notes to the fee calculation sheet

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

eiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 7024473P118

Box No. I TITLE OF INVENTION

METHODS AND COMPOSITIONS FOR REGULATING DEVELOPMENTAL IDENTITY

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PURDUE RESEARCH FOUNDATION
Office of Technology Commercialization
1291 Cumberland Avenue
West Lafayette, Indiana 47906 US

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

OGAS, Joseph P.
805 N. Chauncey Avenue
West Lafayette, Indiana 47906 US

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHWARTZ, Jason J.
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
Bank One Center/Tower, Suite 3700
111 Monument Circle
Indianapolis, Indiana 46204 US
SEE CONTINUATION TO BOX NO. III ON SHEET NO. 4

Telephone No. 317-634-3456

Facsimile No. 317-637-7561

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2 ..... Agent's : 7024473P118

Continuation of Box No. III [redacted] **RTHER APPLICANT(S) AND/OR (FURTHE** [redacted] **VENTOR(S)**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SOMERVILLE, Chris R.
5 Valley Oak
Portola Valley, California 94028 US

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATI F STATES

The following designations ar by made under Rule 4.9(a) *(mark the applicable check xes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **MZ** Mozambique, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AE** United Arab Emirates
- ☒ **AG** Antigua and Barbuda
- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria .....
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus .....
- ☒ **BZ** Belize
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CR** Costa Rica .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic .....
- ☒ **DE** Germany .....
- ☒ **DK** Denmark .....
- ☒ **DM** Dominica
- ☒ **DZ** Algeria .....
- ☒ **EE** Estonia .....
- ☒ **ES** Spain .....
- ☒ **FI** Finland .....
- ☒ **GB** United Kingdom
- ☒ **GD** Grenada
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **GM** Gambia
- ☒ **HR** Croatia .....
- ☒ **HU** Hungary .....
- ☒ **ID** Indonesia
- ☒ **IL** Israel .....
- ☒ **IN** India .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakhstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MA** Morocco .....
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **MZ** Mozambique
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia .....
- ☒ **SL** Sierra Leone .....
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **TZ** United Republic of Tanzania
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZA** South Africa .....
- ☒ **ZW** Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

Continuation to Box No. IV Agent

WOODARD, Harold R.; EMHARDT, C. David; NAUGHTON, Joseph A., Jr.; MORIARTY, John V.; McNETT, John C.; HENRY, Thomas Q.; DURLACHER, James M.; REEVES, Charles R.; WAGNER, Vincent O.; ZLATOS, Steve; BEREVESKOS, Spiro; BAHRET, William F.; BROWNING, Clifford W.; FRISK, R. Randall; LUEDERS, Daniel J.; GANDY, Kenneth A.; THOMAS, Timothy N.; SISSELMAN, Kerry P.; JONES, Kurt N.; ALLIE, John H.; BANTA, Holiday W.; COLE, Troy J.; PAYNTER, L. Scott; LOWES, J. Andrew; MEYER, Charles J.; HARRIS, Darrin Wesley; SCHANTZ, Matthew R.; COY, Gregory B.; HIDAY, Lisa A.; DANILUCK, John V.; BROWN, Christopher A.; BRANNON, C. John; SCHWARTZ, Jason J.; USHER, Arthur J. IV; COLLIER, Douglas A.; SCHEPERS, Brad A.; TUCKER, R. Craig; STEVENS, Scott J.; MYERS, James B. Jr.; and ROWE, James L., all of Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, Indiana 46204 United States of America

Box No. VI PRIORITY C ☐ Further priority [redacted]s are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (20.08.99) 20 August 1999	60/149,975	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)* :
ISA/ US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date (day/month/year)	Number	Country (or regional Office)
20 August 1999	(20.08.99)	60/149,975 US

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	5
description (excluding sequence listing part)	:	49
claims	:	12
abstract	:	1
drawings	:	4
sequence listing part of description	:	21
Total number of sheets	:	92

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter (dup)

Figure of the drawings which should accompany the abstract: NONE

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Applicant(s):
PURDUE RESEARCH FOUNDATION
OGAS, Joseph P.
SOMERVILLE, Chris R.

Agent:

(Jason J. SCHWARTZ)

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14519 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US00/22725

(22) International Filing Date: 18 August 2000 (18.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/149,975 20 August 1999 (20.08.1999) US

(71) Applicant *(for all designated States except US)*: **PURDUE RESEARCH FOUNDATION** [US/US]; Office of Technology Commercialization, 1291 Cumberland Avenue, West Lafayette, IN 47906 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **OGAS, Joseph, P.** [US/US]; 805 N. Chauncey Avenue, West Lafayette, IN 47906 (US). **SOMERVILLE, Chris, R.** [US/US]; 5 Valley Oak, Portola Valley, CA 94028 (US).

(74) Agents: **SCHWARTZ, Jason, J.** et al.; Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/14519 A2

(54) Title: METHODS AND COMPOSITIONS FOR REGULATING DEVELOPMENTAL IDENTITY

(57) Abstract: Purified PKL proteins that function in regulating developmental identity in host cells are provided. Nucleotide sequences encoding functional PKL proteins are also provided. The invention also provides recombinant vectors including the nucleotide sequences encoding PKL, eukaryotic host cells and transgenic plants that include the introduced nucleotide sequences described herein, and methods of transforming plants utilizing the constructs described herein.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14519 A3

(51) International Patent Classification[7]: C12N 5/04, 15/00, 15/12, 15/29, 15/63, 15/64, 15/82, 15/85, 15/87, 15/90, A01H 5/00, C07H 21/02, 21/04

(21) International Application Number: PCT/US00/22725

(22) International Filing Date: 18 August 2000 (18.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/149,975 20 August 1999 (20.08.1999) US

(71) Applicant *(for all designated States except US)*: **PURDUE RESEARCH FOUNDATION** [US/US]; Office of Technology Commercialization, 1291 Cumberland Avenue, West Lafayette, IN 47906 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **OGAS, Joseph, P.** [US/US]; 805 N. Chauncey Avenue, West Lafayette, IN 47906 (US). **SOMERVILLE, Chris, R.** [US/US]; 5 Valley Oak, Portola Valley, CA 94028 (US).

(74) Agents: **SCHWARTZ, Jason, J.** et al.; Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
30 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/14519 A3

(54) Title: METHODS AND COMPOSITIONS FOR REGULATING DEVELOPMENTAL IDENTITY

(57) Abstract: Purified PKL proteins that function in regulating developmental identity in host cells are provided. Nucleotide sequences encoding functional PKL proteins are also provided. The invention also provides recombinant vectors including the nucleotide sequences encoding PKL, eukaryotic host cells and transgenic plants that include the introduced nucleotide sequences described herein, and methods of transforming plants utilizing the constructs described herein.

INTERNATION SEARCH REPORT

[redacted]ational application No.
PCT/US00/22725

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 320.2, 410, 419, 455, 468, 471; 536/23.1, 23.5, 23.6; 800/21, 278, 286, 287, 290, 295, 298

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West, Agricola, Medline, Caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	OGAS et al. PICKLE Is A CHD3 Chromatin-Remodeling Factor That Regulates The Transition From Embryonic To Vegetative Development In Arabidopsis. Proc. Natl. Acad. Sci. USA. 23 November 1999. Vol. 96. No. 24. pages 13839-13844, see whole document.	1-83
X --- Y	JIN et al. Isolation And Characterization Of Hrp1+, A New Member Of The SNF2/SWI2 Gene Family From The Fission Yeast Schizosaccharomyces pombe. Mol. Gen. Genet. 1998. Vol. 257. pages 319-329, especially pages 321-324, 326-327.	58, 63, 64, 67, 69, 70, 71, 74, 80 --- 1, 3-15, 21-23, 25, 26, 30-33, 37, 40, 42, 44, 45, 49, 52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 FEBRUARY 2001	19 MAR 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ASHWIN MEHTA TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1800
Facsimile No. (703) 305-3230	Telephone No. (70[redacted]

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIO SEARCH REPORT

[redacted]ational application No.
PCT/US00/22725

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WOODAGE et al. Characterization Of The CHD Family Of Proteins. Proc. Natl. Acad. Sci. USA. October 1997. Vol. 94. pages 11472-11477, see whole document.	58, 63, 64, 67, 69-71, 74, 80 ---------- 1-15, 21-26, 30-33, 37, 40, 42, 44, 45, 49, 52
X ---- Y	STOKES et al. CHD1 Is Concentrated In Interbands And Puffed Regions Of Drosophila Polytene Chromosomes. Proc. Natl. Acad. Sci. USA. July 1996. Vol. 93. pages 7137-7142, see pages 7138-7141.	58, 63, 64, 67, 69, 70, 71, 74, 80 ---------- 1, 3-14, 21-26, 30-33, 37, 40, 42, 44, 45, 49, 52
X --- Y	DELMAS et al. A Mammalian DNA-Binding Protein That Contains A Chromodomain And An SNF2/SWI2-Like Helicase Domain. Proc. Natl. Acad. Sci. USA. March 1993. Vol. 90. pages 2414-2418, especially pages 2415, 2416, 2418.	58, 63, 64, 67, 69, 70, 71, 74, 80 ---------- 1, 3-14, 21-26, 30-33 37, 40, 42, 44, 45, 49, 52

INTERNATIO SEARCH REPORT

[redacted]national application No.
PCT/US00/22725

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C12N 5/04, 15/00, 15/12, 15/29, 15/63, 15/64, 15/82, 15/85, 15/87 15/90; A01H 5/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 320.2, 410, 419, 455, 468, 471; 536/23.1, 23.5, 23.6; 800/21, 278, 286, 287, 290, 295, 298

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group i, claim(s)1-15, 18-37, 55-75, and 77-79, drawn to a first method of transforming any host cell, comprising introducing into any host cell a nucleic acid molecule encoding a protein having at least one chromo domain, a helicase domain, and a DNA binding domain, or wherein said protein encodes PKL; a first product a recombinant nucleic acid molecule comprising said nucleic acid molecule and a foregin promoter, and a transgenic plant comprising said nucleic acid molecule.
Group II, claim(s) 16 and 17, drawn to a second method, of transforming any host cell, comprising introducing into any host cell a nucleic acid molecule encoding a protein having a point mutation in lysine 304.
Group III, claim(s) 38-54 and 76, drawn to a third method, of transforming any host cell, comprising introducing into any host cell an antisense DNA or RNA molecule; and a second product, a transgenic plant comprising said antisense DNA or RNA molecule.
Group IV, claims 80-83, drawn to a third product, a recombinant protein, and a fourth method, of producing a PKL protein.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of Group I does not share the non-mutated nucleic acid molecule with Group II, III, or IV. The mutant sequence of Group II is not share with any of the other groups. The antisense molecules of the method of Group III is not shared with the method nucleotide sequences of Group I, the mutant molecule of Group II, nor the protein and method of Group IV. The protein of Group IV is not shared with any of the other groups.

PATENT COOPERATION TREATY

RECEIVED

NOV 1 2 2001

Woodard, Emhardt, Naughton, Moriarty & McNett

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: JASON J. SCHWARTZ
WOODARD, EMHARDT, NAUGHTON, MORIARTY
& MCNETT
BANK ONE CENTER/TOWER, SUITE 3700
111 MONUMENT CIRCLE
INDIANAPOLIS, INDIANA 46204

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	06 NOV 2001

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
7024473P118	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US00/22725	18 AUGUST 2000	20 AUGUST 1999

Applicant
PURDUE RESEARCH FOUNDATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ASHWIN MEHTA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: JASON J. SCHWARTZ
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
BANK ONE CENTER/TOWER, SUITE 3700
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INDIANAPOLIS, INDIANA 46204

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Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ASHWIN MEHTA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 7024473P118	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/22725	International filing date *(day/month/year)* 18 AUGUST 2000	Priority date *(day/month/year)* 20 AUGUST 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant PURDUE RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 18 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [X] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [X] Certain defects in the international application

   VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19 MARCH 2001	19 OCTOBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ASHWIN MEHTA Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/22725

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached), as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:
pages (See Attached), as originally filed
pages _____, as amended (together with any statement) under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:
pages (See Attached), as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached), as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE
☒ the claims, Nos. NONE
☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

ernational application No.
PCT/US00/22725

IV. Lack of unity of inventi n

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [X] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-37, 55-57, and 77-79, drawn to a first method of transforming any host cell, comprising introducing into said cell a nucleic acid molecule encoding a protein having at least one chromo domain, a helicase domain, and a DNA binding domain, or wherein said protein encodes PKL; a first product- a recombinant nucleic acid molecule comprising said nucleic acid molecule and a foreign promoter, and a transgenic plant comprising said nucleic acid molecule.
Group II, claim(s) 38-54, and 76, drawn to a second method, of transforming any host cell, comprising introducing into any host cell an antisense DNA or RNA molecule; and a second product, a transgenic plant comprising said antisense DNA or RNA molecule.
Group III, claim(s) 80-83, drawn to a third product, a recombinant protein, and a third method, of producing a PKL protein.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of Group I does not share the nucleic acid molecule with Group II or III. The antisense molecules of the method of Group II are not shared with the method or nucleotide sequences of Group I, nor the protein and method of Group III. The protein of Group III is not shared with any of the other groups.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. _.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

ernational application No.
PCT/US00/22725

V. Reas ned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. citations and explanations (Rule 70.7)

Claims 1, 4-10, 20-23, 26-27, 30, 33, 35, 37, 55, 58-60, 63-65, 67, 70, 71, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Jin et al.

Jin et al teach the isolation and characterization of the hrp1+ gene. It contains a chromodomain, a helicase domain, and a DNA-binding domain, and acts as a negative regulator of cell growth. Jin et al also teach overexpression of hrp1+ in yeast cells (pages 321-324, 326).

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants concede that hrp1+ may produce a gene involved in gene expression generally and that its function may relate to cell growth, but argue that Jin et al do not teach or suggest hrp1+ being involved in developmental identity. Applicant's arguements have been fully considered but were not found persuasive. As hrp1+ is a yeast gene whose product is involved in regulating cell growth, it can be considered as being involved in the regulation of development of yeast cells. As written, the claimed invention is anticipated by Jin et al.

Claims 55-60, 63-65, 67-69, 80, and 81 lack novelty under PCT Article 33(2) as being anticipated by Woodage et al.

Woodage et al teach the characterization of CHD family of proteins. Numerous proteins having chromo domains, helicase domains, and DNA binding domains are taught, for example the murine CHD1 protein, and the gene which encodes it (page11472-11474). CHD proteins which further contain a zinc-finger domain and/or a second chromo domain are also taught, for example HsCHD3 (pages 11473 and 11475). Yeast strains transformed with ScCHD1 are also taught (page 11473).

Applicants traverse the rejection in the paper submitted 28 August 2001. Applicants argue that Woodage et al do not teach or suggest a nucleic acid sequence that codes for a protein that has the recited domains and functions to regulate developmental identity. Applicant's arguements have been fully considered but were not found persuasive. That the proteins taught by Woodage et al have function in regulating developmental identity would be property inherent to them.
(Continued on Supplemental Sheet.)

VII. Certain def cts in the international application

The following defects in the form or contents of the international application have been noted:

Claims 2 and 43 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: The claims are exactly identical.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that the claims are dependent on different independent claims, of different scope. However, both claims 2 and 43 are dependent on claim 1.

VIII. Certain bservations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 4-9, 20, 26, 27, 33-35,37,40-42, 45-47, 49-52, 54, 58-61, 63-65, 70, 71, 73-78, 80, 81, and 83 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): The recitations "at least about" and "about" render the claims indefinite. The boundaries of the indicates nucleotide sequences are not made clear by the recitations, and therefore the metes and bounds of the claims are unknown.

Claims 56 and 57 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): Dependent claims 56 and 57 refer to the "method of claim 55", which is drawn to a product.

Claims 18, 24, 47, 78 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the term "PKL" renders the claims indefinite. The description at page 10, lines 24-26 indicates that all of the described proteins are generally referred to as "PKL". It is therefore not clear which particular PKL protein the claims are referring to.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that one skilled in the art would clearly understand the term "PKL" in light of the description, citing for example that SEQ ID NO: 2 shows one preferred embodiment of PKL, and that variants of the polypeptide are included as described on page 10, and that a description may also be found on pages 11-13. Applicant's arguements have been fully considered but were not found persuasive. The description does not define how PKL is distinguished from other genes encompassed by claim 1. Further "PKL" appears to be an arbitrary designation. It is not clear how one would identify another PKL if others in the art refer to homologs by another designation.

Claim 24 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): There is no antecedent basis for "PKL" in the claim or parent claim 1.

Applicants traverse the objection in the paper submitted 28 August 2001. Applicants argue that amended claim 24 is now dependent on claim 18. However, there is still no antecedent basis for "said plant" in claim 24 or in the claims from which it depends.

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 5/04, 15/00, 15/12, 15/29, 15/63, 15/64, 15/82, 15/85, 15/87 15/90; A01H 5/00; C07H 21/02, 21/04 and US Cl.: 435/69.1, 320.2, 410, 419, 455, 468, 471; 536/23.1, 23.5, 23.6; 800/21, 278, 286, 287, 290, 295, 298

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-9, 13-23, 25, 27-30, 32-41, 49, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 10-12, 24, 26, 31, and 42-48, filed with the letter of 28 August 2001.

This report has been drawn on the basis of the claims,
page(s) 50, 54-56, 59, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 51-53, 57, 58, 60, and 61, filed with the letter of 28 August 2001.

This report has been drawn on the basis of the drawings,
page(s) 1-4, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
Pages 1-28, filed wit the letter of 28 August 2001.

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The report as to Novelty was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The report as to Inventive Step was positive (YES) with respect to claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, 83.
The report as to Inventive Step was negative (NO) with respect to claims 1, 4-10, 20-23, 26, 27, 30, 33, 35, 37, 55-60, 63-65, 67-71, 80, 81.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-82.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Claims 2, 3, 11-19, 24, 25, 28, 29, 31, 32, 34, 36, 38-54, 61, 62, 66, 72-79, 82, and 83 meet the criteria set out in PCT Article 33(2) and (3), because the prior art does not teach or fairly suggest SEQ ID NO: 1, the Arabidopsis PKL gene or protein, a method of transforming a host cell with any of the claimed nulceotide sequences which include sequences encoding a zinc finger or a second chromo domain, or wherein the host cell is an animal or plant cell. Claims 1-82 meet the criteria set out in PCT Article 33(4) in that the claimed nucleotide sequences may be used to control the development of agriculturally important organisms.

---------- NEW CITATIONS ----------

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

NONE

VIII. CERTAIN OBSERVATIONS ON THE APPLICATION (Continued):

Claim 25 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): the recitation "substantial similarity" renders the claim indefinite. It is not clear what is meant by this recitation.

The description is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 5 because it fails to adequately enable practice of the claimed invention because: it does not enable the claimed method when applied to non-plant organisms. The description teaches the PKL protein of Arabidopsis, that it is a "CHD" protein, and a method of expressing it in plants (pages 25-49). Ogas et al teach that PKL may function to establish repression of embryonic identity during germination, and is responsive to the presence of gibberellin (page 13839). Ogas et al also teach that it remains to be determined whether other CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events. At the time the application was filed, therefore, it was not known whether animal CHD proteins could be used in methods to regulate development of host cells. It is therefore unpredictable that the claimed method can regulate developmental identity of all hos cells. It is clear even to the uninitiated that plant systems are quite different from animal systems, and that a role of PKL in plant germination cannot be extrapolated to a role in animal development. In the absense of further guidance, undue experimentation would be required by one skilled in the art to use the claimed method to regulate the development of all host cell types.

Claims 1-10, 12-30, 32-69 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the immediately preceding paragraph.

refers generally to the identity of a tissue during, or at a stage of, development that is brought about by expression of selected genes. For example, selected genes may be expressed in a plant that gives rise to embryonic roots, and thus the developmental identity of the root is embryonic. Furthermore, selected genes may be expressed in a plant that gives rise to seedling roots, and thus the developmental identity of the root is seedling. With specific reference to PKL in pickle roots, one or more genes that gives rise to embryonic roots and one or more genes that gives rise to seedling roots are expressed simultaneously, thus the developmental identity of the root is both embryonic and seedling. The polypeptides described herein are substantially pure (i.e., the proteins are essentially free, e.g., at least about 95% free, from other proteins with which they naturally occur). In one preferred embodiment, the amino acid sequence of a PKL protein having the domains described above, originally found in *Arabidopsis thaliana*, is set forth in SEQ ID:1.

Although the invention is described with reference to *Arabidopsis thaliana* amino acid sequences, it is understood that the invention is not limited to the specific amino acid sequence set forth in SEQ ID:1. Skilled artisans will recognize that, through the process of mutation and/or evolution, polypeptides of different lengths and having differing constituents, e.g., with amino acid insertions, substitutions, deletions, and the like, may arise that are related to, or sufficiently similar to, a sequence set forth herein by virtue of amino acid sequence homology and advantageous functionality as described herein. The term "PKL protein" is used to refer generally to a protein having the features described herein and a preferred example includes a polypeptide having the amino acid sequence of SEQ ID NO:1. Also included within this definition, and in the scope of the invention, are variants of the polypeptide which function in regulating developmental identity, as described herein. Preferred proteins are recombinant proteins.

It is well known that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and yet which effectively provide similar function. For example, an amino acid sequence isolated from another species may differ to a certain degree from the sequence set forth in SEQ ID NO:1, and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although not being limited by theory, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have good activity, even where alterations exist in other portions thereof.

In this regard, a variant of the multi-domain protein described herein, such as a PKL protein variant, is expected to be functionally similar to that set forth in SEQ ID NO:1, for example, if it includes amino acids which are conserved among a variety of species or if it includes non-conserved amino acids which exist at a given location in another species that expresses a functional PKL protein.

Another manner in which similarity may exist between two amino acid sequences is where a given amino acid of one group (such as a non-polar amino acid, an uncharged polar amino acid, a charged polar acidic amino acid or a charged polar basic amino acid) is substituted with another amino acid from the same amino acid group. For example, it is known that the uncharged polar amino acid serine may commonly be substituted with the uncharged polar amino acid threonine in a polypeptide without

substantially altering the functionality of the polypeptide. If one is unsure whether a given substitution will affect the functionality of the enzyme, then this may be determined without undue experimentation using synthetic techniques and screening assays known in the art.

The invention therefore also encompasses amino acid sequences similar to the amino acid sequences set forth herein that have at least about 30% identity thereto and function in regulating developmental identity. Preferably, inventive amino acid sequences have at least about 50% identity, further preferably at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to these sequences.

In preferred embodiments, the invention also encompasses amino acid sequences similar to the amino acid sequences making up polypeptides having the domains described herein. For example, the invention encompasses amino acid sequences that have at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to a first chromo domain from amino acid 115 to amino acid 151 or a second chromo domain extending from amino acid 191 to amino acid 227, at least about 50%, preferably at least about 70%, and more preferably at least about 90% identity to a helicase domain extending from amino acid 293 to amino acid 739, and at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to a DNA binding domain extending from amino acid 1069 to amino acid 1095, and combinations thereof, all as set forth in SEQ ID NO:1. The invention further encompasses amino acid sequences, in addition to those amino acid sequences described above, that have at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to the zinc finger domain amino acid sequence from amino acid 49 to amino acid 96.

Percent identity may be determined, for example, by comparing sequence information using the advanced BLAST computer program, version 2.0, available from the National Institutes of Health. The BLAST

binding domain, or other protein as described herein, such as one having an amino acid sequence having the selected percent identities to the various domains in SEQ ID NO:1 as described herein, including the zinc finger domain. The cell is typically cultured for a time period and under conditions effective for hybridization of the antisense nucleic acid sequence to nucleic acid of the host. The antisense nucleic acid sequence may be DNA or RNA. The length of nucleotides the antisense nucleotide sequence may be complementary to is typically a length sufficient for hybridization to the target nucleic acid sequence so that transcription and/or translation will be substantially inhibited and/or production of a functional protein will be substantially stopped or otherwise substantially decreased. For example, antisense nucleotide sequence may be at least about 25 nucleotides long, and may further be about 50 to about 4200 nucleotides long, preferably about 100 to about 1000 nucleotides long, and further more preferably about 200 to about 500 nucleotides long. In preferred forms of the invention, the antisense nucleic acid sequence may be complementary to, for example, a region from about nucleotide 2 to about nucleotide 331 set forth in SEQ ID NO:1. In other preferred forms of the invention, the antisense nucleic acid sequence may be complementary to a region from about nucleotide 3330 to about nucleotide 3710 in SEQ ID NO:1.

In yet another form of a method of transforming a host cell, a method may include introducing into the host cell a vector that includes a nucleic acid molecule that may be used to generate a nucleic acid molecule, such as an antisense RNA molecule, that will bind to the endogenous transcript in order to inhibit translation of the transcript and to target the transcript for degradation. In one form, the method may include introducing into the host cell a vector that includes length of nucleotides within the nucleotide sequence shown in SEQ ID NO:1 along with the same nucleotides in an antisense orientation. As an example, the host cell may be transformed with a construct that includes, in the following order, a promoter, operably linked to, for example, a PKL fragment as described herein in the sense

Ecker, J. R. (1994) *Genomics* 19:137-144]. The AFLP analysis was performed as described by Liscum, E. and Oeller, P. W. (1999) *Genome Analysis, P. Offner* (Ed.),. CRC Press, Boca Raton, FL, in press]. The AFLP primers used for mapping analysis were as follows: the basic EcoRI primer is 5'-AGA CTG CGT ACC ATT TCx y-3' (where x and y indicate base pairs added for specificity), shown in SEQ ID NO:2, and the basic MseI primer is 5'-GAT GAG TCC TGA GTA Axy z-3' (where x, y, and z indicate base pairs added for specificity), shown in SEQ ID NO:3. E11M48 denotes the primer pair EcoRI-AA and MseICAC, E11M49 denotes the primer pair EcoRI-AA and MseI-CAG, and EI4M59 denotes the primer pair EcoRI-AT and MseICTA [Alonso-Blanco, C. et al. (1998) *Plant J.* 14: 259-271].

To identify polymorphisms in the fast neutron-derived alleles of *PKL*, Southern blots were performed using genomic DNA from plants and digoxigenin-labeled probes that were generated from YAC DNA using AFLP technology. DNA from YAC CIC8H12 (YAC CIC8H12 was obtained from the Arabidopsis Biological Resource Center, Columbus, Ohio) and was prepared as described [Gibson, S. I. and Somerville, C. (1992) *World Scientific:* 119-143]. Approximately 50 ng of CIC8H12 DNA was utilized in a restriction and ligation reaction as described at http://carnegiedpb.stanford.edu/methods/aflp.htmi, with the following differences: the DNA was only digested with MseI, and only the MseI adaptor was ligated on. Five µl of this restriction and ligation (RAL) mixture was then used in a 100 µl digoxigenin-labeling PCR reaction (Roche Biochemicals, cat. # 1 636 090) with 100 pmol each of 6 MseI-xy primers (where x and y indicate base pairs added for specificity). The entire PCR reaction was then used to probe a Southern blot as described in the Dig User's Guide (Roche Biochemicals, cat. # 1 438 425). Random combinations of 6 MseI-xy primers were used to screen for polymorphisms in the fast neutron-derived alleles. Polymorphisms were revealed when the following 6 primers were utilized: xy = CT, GG, GC, AG, TG, AT.

EXAMPLE 2

Characterization of PKL

Ribonuclease protection assays.

Ribonuclease protection assays were performed using the RPA III kit from Ambion (cat. # 1414). To generate a PKL-specific probe, a DNA fragment was generated via RT-PCR using the primers JOpr244 (5'-TGT TGA GCC AGT TAT TCA CGA-3'), (nucleotides 1725-1745 in SEQ ID NO:1) shown in SEQ ID NO:4, and JOpr247 (5'-ACC TTT CCA TCA ATT CGC TCG-3') (sequence complementary to nucleotides 1934-1914 in SEQ ID NO:1) shown in SEQ ID NO:5, and subcloned using the pGEM-T vector system (Promega, cat. # A3600) in an orientation such that the T7 promoter would produce an anti-sense transcript. This plasmid was called pJ0657. To generate a *LEC1*-specific probe, a DNA fragment was generated via PCR using the primers JOpr273 (5'CCGCTCGAGAACCCCAATGACCAGCTCAGT-3'), shown in SEQ ID NO:6 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 33-53 of LEC1 cDNA sequence, Genbank Accession No. AF036684), and JOpr262 (5'-CCTTCTTCACTTATACTGACC-3'), shown in SEQ ID NO:7 (sequence complementary to nucleotides 672-652 of LEC1 cDNA sequence, Genbank Accession No. AF036684), digested with XhoI and KpnI and subcloned into pBluescript SK cut with XhoI and KpnI to produce pJ0660. To generate a ROC3-specific probe, a DNA fragment was generated via PCR using the primers JOpr276 (5'-AAGTCTACTTCGACATGACCG-3'), shown in SEQ ID NO:8 (nucleotides 65-85 of ROC3 cDNA sequence, Genbank Accession No. U40399), and JOpr277 (5'-CTTCCAGAGTCAGATCCAACC-3'), shown in SEQ ID NO:9 (sequence complementary to nucleotides 524-504 of ROC3 cDNA sequence, Genbank Accession No. U40399), and subcloned using the pGEM-T vector system in an orientation such that the T7 promoter would produce an anti-sense transcript. This plasmid was called

In conclusion, cloning of a gene necessary for repression of embryonic identity has lead to the proposition that a GA-modulated chromatin remodeling factor mediates a developmental transition in *Arabidopsis*. It is anticipated that further characterization of *PKL*, and identification of proteins that either regulate or are targets of *PKL*, will shed light on the mechanism of GA signal transduction and the role of GA in regulating differentiation and development in *Arabidopsis*. It remains to be determined whether CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events.

EXAMPLE 4

Generation of Mutant PKL by a Dominant Negative Strategy

It has previously been demonstrated that a point mutation of a conserved lysine in the ATPase/helicase domain of SWI/SNF proteins generates a dominant negative mutant form of the protein [Chavari et al., (1993) *Nature* 366:170-174). By mutating the analogous mutation in PKL (by mutating Lys-304 to an Arg residue), a dominant negative version of PKL may be generated. This mutant allele of *PKL* may be generated by a PCR strategy.

A complementation construct for PKL was generated that includes the PKL cDNA flanked by 1.1 kb of upstream genomic sequence (to the BstBI site) and 1.4 kb of downstream genomic sequence (to the NcoI site). The construct was generated by performing overlap PCR on PKL cDNA with three DNA fragments: the genomic fragment upstream of the PKL start codon to the BstBI site, the PKL cDNA and the genomic fragment downstream of the termination codon to the NcoI site. A BstBI – XhoI fragment (2.1 kb) from this construct has been subcloned into a modified pBluescript vector (pJO674). The modified pBluescript vector pJ0674 was formed by ligating in a cassette generated by annealing the primers JOpr386 (5'-CTTCGAACTCGAGGGATCCCCATGGCTAGCAGCT-3'), shown in SEQ ID NO:25 (this is a synthetic sequence that includes "A"

followed by the recognition sequence of BstB1, XhoI, Bam HI, NcoI, Nhe I and sequence "AGCT" wherein the last "G" in the NcoI recognition sequence and the first "G" in the NheI recognition sequence overlap) and JOpr387(5'-GCTAGCCATGGGGATCCCTCGAGTTCGAAGGTAC), as shown in SEQ ID NO:26 (this is a synthetic sequence complementary to SEQ ID NO:25) after pBluescript was cut with KpnI and SacI. The resulting cassette include the following restriction sites: BstB1, XhoI, Bam HI, NcoI and NheI. 2 separate PCR reactions have been performed using this vector as a substrate. 1 PCR reaction uses a T3 primer with the following primer shown in SEQ ID NO:10 (JOpr516) 5'-GAAATGGGACTAGGCAGGACAATTCAAAGC-3' (nucleotides 895-924 in SEQ ID NO:1) where the underlined G is designed to replace an A residue in the wild-type PKL sequence and introduce the Lys-304 to Arg-304 mutation. This reaction generates a 272 bp fragment. The other PCR reaction uses a T7 primer with the following primer shown in SEQ ID NO:11 (JOpr517) 5'-GCTTTGAATTGTCCTGCCTAGTCCCATTTC-3' (sequence complementary to SEQ ID NO:1 from nucleotides 924-895) where the underlined C is designed to replace a T residue in the wild-type PKL sequence and introduce the Lys-304 to Arg-304 mutation. This reaction generates a 2094 bp fragment. Overlap PCR can then be done by adding the 272 bp and 2094 bp fragment together along with the T3 and T7 primers generating a 2.3 kb fragment. This fragment will be digested with BstBI and XhoI, cloned back into pJO674 and then sequenced to verify introduction of the mutation. This vector will then be cut with BstBI and XhoI and ligated into a pBluescript-based vector carrying the complementation construct (pJO765, formed by ligating the complementation fragment into pJO674 cut with BstBI and NcoI) cut with BstBI and XhoI, resulting in generation of a complementation construct that carries the dominant negative mutation. This construct will then be transferred to a binary vector [a modified pCAMBIA3300, pJO630, which is formed by digesting pCAMBIA3300 with BstXI and EcoRI and ligating in the

cassette generated by annealing primers JOpr232 (5'-CCAGGTACCTGG-3'), shown in SEQ ID NO:27 and JOpr233 (5'-AATTCCAGGTACCTGGCATG-3'), shown in SEQ ID NO:28] and transformed into wild-type plants to verify generation of a mutant *pkl* phenotype. These sequences are synthetic sequences that anneal to form a cassette that has ends that are compatible to BstXI and EcoRI digested pCAMBIA3300. The entire sequence of JOpr232 is a new site that when cut with BstXI generates ends that are compatible with KpnI ends. The cassette thus recreates a BstXI site with KpnI compatible ends. The PCR reactions and subcloning are performed as known in the art, and as described, for example, in Sambrook et al. (Eds.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989).

A conditional version of this dominant negative allele may be made by fusing the gene to the glucocorticoid receptor [Lloyd et al., (1994) *Science* 266:436-439). A clone of the rat glucocorticoid receptor (GR) was obtained from Alan Lloyd, at the University of Texas, Austin, Texas. The clone included SEQ ID NO:29 (5'-TCTAGAGGATCCTGAAGCTCGAAAAACAAAGAAAAAAA-3'), that is fused to nucleotides 1569-2407 of rat glucocorticoid receptor cDNA found in Genbank Accession No. Y12264. SEQ ID NO:29 was used to add spacers and restriction sites to the clone. A PCR reaction has been performed with this GR clone as a substrate and the following primers: JOpr533 (5'-AAGCCAAAGAACATGGTCGTTGATCTAGAGGATCCTGAAGCTCGAAA-3') shown in SEQ ID NO:12 (the first 24 nucleotides are nucleotides 4129-4152 of SEQ ID NO:1 whereas the last 23 nucleotides are nucleotides 2-24 of SEQ ID NO:29 of the rat glucocorticoid receptor cDNA found in Genbank Accession No. Y12264) and JOpr534 (5'-GAATCTTGATTTACCAGTTGAGTCATTTTTGATGAAACAGAAGCTTTTT GAT-3') (the first 25 nucleotides are nucleotides complementary to nucleotides 4153-4177 of SEQ ID NO:1 and the last 27 nucleotides are

complementary to nucleotides 2407-2381 of the glucocorticoid receptor cDNA found in Genbank Accession No. Y12264) shown in SEQ ID NO:13, which are designed to add PKL sequences to the end of the GR fragment such that overlap PCR can be performed. A BamHI –NcoI fragment of the complementation construct has been subcloned into pJO674, generating vector pJO724. pJO724 may be the substrate for 2 PCR reactions. One reaction can use the T7 primer and JOpr398 (5'-ATCAACGACCATGTTCTTTGG-3') (sequence complementary to nucleotides 4152-4132 of SEQ ID NO:1), shown in SEQ ID NO:14, generating a 883 bp fragment. The other reaction will use the T3 primer and JOpr401 (5'- TGACTCAACTGGTAAATCAAGA-3') (nucleotides 4153-4174 of SEQ ID NO:1), shown in SEQ ID NO:15, generating a 1.5 kb fragment. Overlap PCR can then be performed using 883 bp fragment and the GR fragment with the T7 primer and JOpr534. Overlap PCR can then be performed again using the product of this PCR reaction and the 1.5 kb fragment using the T7 primer and the T3 primer. This PCR product can then be digested with BamHI and NcoI and cloned back into pJO674 digested with the same. The construct will then be sequenced to verify identity. This construct can then be digested with BamHI and NcoI and ligated to the dominant-negative version of the complementation construct to generate a C-terminal fusion of GR to the mutant PKL protein. Once again, this construct can be transferred to a binary vector (pJO630) and transformed into wild-type plants to verify that a mutant *pkl* phenotype will be generated upon addition of dexamethasone.

If necessary, the dominant-negative version of the gene may be overexpressed in order to generate a phenotype. In this case, the mutated ORF (+/- GR) can be cloned downstream of a constitutive high level promoter such as the 35S promoter in a binary vector.

In all of Examples 4-6 described herein, ribonuclease protection assays will be performed to verify expression of the mutant transcript. The

pkl phenotype will be assayed by penetrance of the pickle root phenotype and by the rosette phenotype [Ogas, J. et al. (1997) *Science* 277:91-94].

EXAMPLE 5

Generation of Mutant PKL by Antisense Procedures

Two constructs for inhibiting expression of endogenous *PKL* by iRNA may be generated. These constructs are based on sequence comparison between PKL and PKR2, which is another CHD protein that exhibits high sequence similarity to PKL. A fragment of PKL may be cloned into the vector pRNA69, which results in formation of the following construct: 35S promoter – *PKL* frag in sense orientation – intron – the same PKL frag in antisense orientation – terminator. Vector pRNA69 is a bacterial vector that was obtained from John Bowman at UC Davis.

The sequence of the *PKL* cDNA that is being targeted in the first construct is from nucleotide 2 to nucleotide 361 in SEQ ID NO:1. This fragment was generated by performing PCR on PKL cDNA with the following primers: JOpr442 (5'-CCGCTCGAGTGAGTAGTTTGGTGGAGAGGC-3') found in SEQ ID NO:16 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 2-22 of SEQ ID NO:1) and JOpr443 (5'-CCGGAATTCCATCGGAGGAACCTTGTTCAC-3'), found in SEQ ID NO:17(the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Eco RI recognition sequence whereas the last 21 nucleotides are complementary to nucleotides 361-341 of SEQ ID NO:1), for the cloning the sense orientation (as a XhoI-EcoRI fragment) and JOpr444 (5'-CGCGGATCCCATCGGAGGAACCTTGTTCAC-3'), shown in SEQ ID NO:18 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI

recognition sequence and the last 21 nucleotides are complementary to nucleotides 361-341 of SEQ ID NO:1) and JOpr445 (5'-TGCTCTAGATGAGTAGTTTGGTGGAGAGGC-3'), shown in SEQ ID NO:19 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XbaI recognition sequence and the last 21 nucleotides are nucleotides 2-22 of SEQ ID NO:1), for cloning the antisense orientation (as a BamHI-XbaI fragment) into pRNA69.

The sequence of the *PKL* cDNA that is being targeted in the second construct is from nucleotide 3330 to nucleotide 3710 in SEQ ID NO:1. This fragment was generated by performing PCR on PKL cDNA with the following primers: JOpr446 (5'-CCGCTCGAGCCCTCACATAAGTTTGTCTGC-3'), shown in SEQ ID NO:20 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 3330-3349 of SEQ ID NO:1), and JOpr447 (5'-CCGGAATTCGTCTTAGGAAGTCCATCAAGC-3'), shown in SEQ ID NO:21 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Eco RI recognition sequence and the last 21 nucleotides are complementary to nucleotides 3710-3690 of SEQ ID NO:1), for the cloning the sense orientation (as a XhoI-EcoRI fragment) and JOpr448 (5'-CGCGGATCCGTCTTAGGAAGTCCATCAAGC-3'), found in SEQ ID NO:22 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI recognition sequence whereas the last 21 bases are nucleotides 3330-3351 in SEQ ID NO:1), and JOpr449 (5'-TGCTCTAGACCCTCACATAAGTTTGTCTGC-3'), shown in SEQ ID NO:23 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XbaI recognition

sequence and the last 21 nucleotides are nucleotides 3330-3350 in SEQ ID NO:1)for cloning the antisense orientation (as a BamHI-XbaI fragment) into pRNA69.

The pRNA69 constructs may then be ligated into the binary vector pBART by making use of the flanking NotI sites. Wild-type plants may then be transformed by these constructs by vacuum infiltration. The plants may then be screened for a mutant pkl phenotype as described for Example 5.

EXAMPLE 6

Generation of Mutant PKL by Domain Deletion

It has been shown that removing the DNA-binding portion of *CHD1* in *S. cerevisiae* generates an inactive form of the protein [Woodage et al., (1997) *PNAS* 94:11472-11477). By specifically deleting the DNA-binding domain (aa 1069 - 1095) or any of the other domains, a dominant negative version of *PKL* may be produced. The XhoI-BamHI fragment of the PKL cDNA sequence has been cloned into pJO687, a vector obtained by introducing this fragment into a pJO674 vector formed as described in Example 4. In order to delete the putative DNA binding domain of PKL, PCR mutagenesis may be used. Briefly, a PCR reaction may be performed using pJO687 as a substrate and T7 and the oligo 5'-CGCGGATCCTTTTTCCACTTCTCAGTCCGGG-3', shown in SEQ ID NO:24 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI recognition sequence and the last 21 nucleotides are complementary to nucleotides 3202-3181 of SEQ ID NO:1), as a primer. The product can be digested with XhoI and BamHI and cloned into pJO674 cut with the same, and then can be sequenced to verify introduction of the mutation. This vector can then be cut with XhoI and BamHI and ligated into a pBluescript-based vector, carrying the complementation construct (pJO765) cut with the same, resulting in generation of a complementation construct that carries PKL deleted for the DNA binding domain. This construct can then

6. The method of claim 3, wherein said second chromo domain is encoded by a nucleic acid molecule having a nucleotide sequence having at least about 50% identity to the nucleotide sequence set forth in SEQ ID NO:1 from nucleotide 571 to nucleotide 681.

7. The method of claim 1, wherein said nucleic acid molecule has a nucleotide sequence encoding protein domains selected from the group consisting of a chromo domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 from amino acid 115 to amino acid 151, a helicase domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 from amino acid 293 to amino acid 739 and a DNA binding domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1069 to amino acid 1095.

8. The method of claim 2, wherein said nucleic acid molecule has a nucleotide sequence encoding said zinc finger domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 from amino acid 49 to amino acid 96.

9. The method of claim 3, wherein said nucleic acid molecule has a nucleotide sequence encoding said second chromo domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 from amino acid 191 to amino acid 227.

10. The method of claim 1, wherein said host cell is a eukaryotic cell.

11. The method of claim 10, wherein said eukaryotic cell is a plant cell.

12. The method of claim 11, wherein said eukaryotic cell is an animal cell.

13. The method of claim 12, wherein said animal cell is a mammalian cell.

14. The method of claim 13, wherein said mammalian cell is a human cell.

15. The method of claim 1, further comprising deleting the nucleotide sequences encoding any one of said domains prior to said introducing.

16. The method of claim 1, wherein said protein has a point mutation in lysine 304.

17. The method of claim 16, wherein said mutation results in said lysine being replaced by an arginine.

18. The method of claim 1, wherein said protein encodes PKL.

19. The method of claim 18, wherein said PKL has an amino acid sequence as set forth in SEQ ID NO:1.

20. The method of claim 1, wherein said nucleic acid molecule has a nucleotide sequence having at least about 80% identity to the nucleotide sequence set forth in SEQ ID NO:1.

21. The method of claim 1, wherein said nucleic acid molecule further comprises a promoter operably linked to a terminal 5' end of said nucleotide sequence.

22. The method of claim 21, wherein said promoter is selected from the group consisting of a constitutive promoter, an inducible promoter and a cell-specific promoter.

23. The method of claim 21, wherein said promoter is a foreign promoter.

24. The method of claim 1, wherein said PKL functions in repressing embryonic identity in said plant.

25. The method of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1.

26. A method of transforming a host cell, comprising introducing into a host cell a nucleic acid molecule encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, said protein functioning in regulating developmental identity.

27. The method of claim 26, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:1.

28. The method of claim 27, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:1.

51. The method of claim 45, wherein said nucleotide sequence is complementary to a region from about nucleotide 3330 to about nucleotide 3710 set forth in SEQ ID NO:1.

52. A method of transforming a host cell, comprising:

(a) introducing into a host cell a vector comprising a first nucleic acid molecule having a nucleotide sequence that is complementary to a nucleotide sequence having at least about 50% identity to a length of nucleotides within the nucleotide sequence set forth in SEQ ID NO:1, said nucleotide sequence encoding a protein functioning in regulating developmental identity;

(b) generating an antisense nucleic acid molecule complementary to an RNA transcript formed from SEQ ID NO:1; and

(b) culturing said host cell under conditions effective for hybridization of said antisense molecule to said RNA transcript of said host cell.

53. The method of claim 52, wherein said nucleic acid molecule has a nucleotide sequence that is complementary to a length of nucleotides within the nucleotide sequence set forth in SEQ ID NO:1.

54. The method of claim 52, wherein the antisense nucleic acid molecule is an RNA molecule.

55. A recombinant nucleic acid molecule, comprising:

(a) a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having at least one chromo domain, a helicase domain and a DNA binding domain, said protein expressible in an amount sufficient to regulate developmental identity.SEQ ID NO:1; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

56. The method of claim 55, wherein said protein further has at least one zinc finger domain.

57. The method of claim 55, wherein said protein further has a second chromo domain.

58. A recombinant nucleic acid molecule, comprising:

(a) a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1 ; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

59. The molecule of claim 58, wherein said foreign promoter is selected from the group consisting of a constitutive promoter, an inducible promoter and a cell-specific promoter.

60. The molecule of claim 58, wherein said protein has an amino acid sequence having at least about 70% identity to the amino acid sequence set forth in SEQ ID NO:1.

61. The molecule of claim 58, wherein said protein has an amino acid sequence of PKL.

62. The molecule of claim 61, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:1.

70. A eukaryotic cell, comprising:

(a) an introduced nucleic acid molecule having a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1.

71. The cell of claim 70, wherein said protein has an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1.

72. The cell of claim 71, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:1.

73. The cell of claim 70, wherein said cell is a plant cell.

74. The cell of claim 70, wherein said cell is an animal cell.

75. A transgenic plant, comprising:

(a) an introduced nucleic acid molecule having a nucleotide sequence encoding a plant protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

76. The transgenic plant of claim 75, wherein said nucleotide sequence is an antisense DNA or RNA molecule that is complementary to said nucleotide sequence.

77. The transgenic plant of claim 75, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:1.

78. The transgenic plant of claim 77, wherein said protein has the amino acid sequence of PKL.

79. The transgenic plant of claim 78, wherein said amino acid sequence is as set forth in SEQ ID NO:1.

80. A recombinant protein, comprising a protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1.

81. The protein of claim 80, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:1.

82. The protein of claim 81, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:1.

83. A method of producing a PKL protein, comprising:

(a) introducing a nucleotide sequence encoding a protein having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1; and

(b) culturing said host cell under conditions effective to achieve expression of the PKL polypeptide.

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Ser Lys Gln Thr His Val Ile Leu Ala Asp Glu Met Gly Leu Gly Lys
    290                 295                 300

aca att caa agc att gcc ctt tta gct tca ctt ttt gag gag aac ctc   960

Thr Ile Gln Ser Ile Ala Leu Leu Ala Ser Leu Phe Glu Glu Asn Leu
305                 310                 315                 320

att ccg cat ttg gta att gct cct cta tcg act ctg cgt aac tgg gag   1008

Ile Pro His Leu Val Ile Ala Pro Leu Ser Thr Leu Arg Asn Trp Glu
                325                 330                 335

aga gag ttt gcc aca tgg gcc cca cag atg aac gtg gtt atg tat ttt   1056

Arg Glu Phe Ala Thr Trp Ala Pro Gln Met Asn Val Val Met Tyr Phe
            340                 345                 350

ggc act gcg caa gct cga gca gtt atc aga gaa cat gag ttt tac tta   1104

Gly Thr Ala Gln Ala Arg Ala Val Ile Arg Glu His Glu Phe Tyr Leu
        355                 360                 365

tcg aaa gat caa aaa aag atc aag aaa aag aaa tct gga caa ata agt   1152

Ser Lys Asp Gln Lys Lys Ile Lys Lys Lys Lys Ser Gly Gln Ile Ser
    370                 375                 380
```

Pklseq1.app

```
agc gaa agc aag caa aaa aga atc aag ttt gat gtc ctc ctc aca tcg   1200

Ser Glu Ser Lys Gln Lys Arg Ile Lys Phe Asp Val Leu Leu Thr Ser
385                 390                 395                 400

tat gag atg atc aac cta gat tca gca gtt cta aaa cca att aag tgg   1248

Tyr Glu Met Ile Asn Leu Asp Ser Ala Val Leu Lys Pro Ile Lys Trp
                405                 410                 415

gag tgc atg att gtt gat gaa ggt cat cga ctg aaa aat aag gat tca   1296

Glu Cys Met Ile Val Asp Glu Gly His Arg Leu Lys Asn Lys Asp Ser
            420                 425                 430

aag ctg ttc tct tca ttg aca cag tat tca agt aac cac cgt att ctt   1344

Lys Leu Phe Ser Ser Leu Thr Gln Tyr Ser Ser Asn His Arg Ile Leu
        435                 440                 445

ctg aca gga aca cca ctt cag aac aac ttg gat gaa ctt ttc atg ctc   1392

Leu Thr Gly Thr Pro Leu Gln Asn Asn Leu Asp Glu Leu Phe Met Leu
    450                 455                 460

atg cat ttt ctt gat gcg ggg aag ttt gga agt ttg gag gag ttc cag   1440

Met His Phe Leu Asp Ala Gly Lys Phe Gly Ser Leu Glu Glu Phe Gln
465                 470                 475                 480

gag gag ttc aaa gat att aat caa gag gag cag atc tca agg ttg cac   1488

Glu Glu Phe Lys Asp Ile Asn Gln Glu Glu Gln Ile Ser Arg Leu His
                485                 490                 495

aaa atg ttg gct cca cat ttg ctc aga agg gta aaa aaa gac gta atg   1536

Lys Met Leu Ala Pro His Leu Leu Arg Arg Val Lys Lys Asp Val Met
            500                 505                 510

aaa gac atg ccc ccc aaa aag gag ctc att ttg cgt gtt gat ctg agc   1584

Lys Asp Met Pro Pro Lys Lys Glu Leu Ile Leu Arg Val Asp Leu Ser
        515                 520                 525

agt ctg cag aaa gaa tat tac aaa gct att ttt acc cgt aat tat caa   1632

Ser Leu Gln Lys Glu Tyr Tyr Lys Ala Ile Phe Thr Arg Asn Tyr Gln
    530                 535                 540
```

Pklseq1.app

```
gta ttg aca aaa aag gga ggt gct caa att tcc ctt aat aac att atg   1680

Val Leu Thr Lys Lys Gly Gly Ala Gln Ile Ser Leu Asn Asn Ile Met
545                 550                 555                 560

atg gaa tta cga aaa gta tgc tgc cat cct tat atg cta gag ggt gtt   1728

Met Glu Leu Arg Lys Val Cys Cys His Pro Tyr Met Leu Glu Gly Val
                565                 570                 575

gag cca gtt att cac gac gca aat gaa gct ttc aaa caa ctt ttg gag   1776

Glu Pro Val Ile His Asp Ala Asn Glu Ala Phe Lys Gln Leu Leu Glu
            580                 585                 590

tct tgt gga aag ctg caa ctt cta gat aaa atg atg gtc aaa ctg aaa   1824

Ser Cys Gly Lys Leu Gln Leu Leu Asp Lys Met Met Val Lys Leu Lys
        595                 600                 605

gag caa gga cac aga gtc cta ata tac aca cag ttt cag cat atg ctg   1872

Glu Gln Gly His Arg Val Leu Ile Tyr Thr Gln Phe Gln His Met Leu
    610                 615                 620

gac tta ctt gaa gac tac tgt acc cat aag aaa tgg cag tac gag cga   1920

Asp Leu Leu Glu Asp Tyr Cys Thr His Lys Lys Trp Gln Tyr Glu Arg
625                 630                 635                 640

att gat gga aag gtt ggc gga gct gag cgg caa ata cgc ata gat cgg   1968

Ile Asp Gly Lys Val Gly Gly Ala Glu Arg Gln Ile Arg Ile Asp Arg
                645                 650                 655

ttc aat gcc aaa aat tct aac aag ttt tgt ttt ttg ctc tcc aca aga   2016

Phe Asn Ala Lys Asn Ser Asn Lys Phe Cys Phe Leu Leu Ser Thr Arg
            660                 665                 670

gct ggt ggc tta gga ata aat ctt gca acg gct gat aca gta atc att   2064

Ala Gly Gly Leu Gly Ile Asn Leu Ala Thr Ala Asp Thr Val Ile Ile
        675                 680                 685

tat gac agt gac tgg aat cct cat gct gat ctt caa gca atg gct aga   2112

Tyr Asp Ser Asp Trp Asn Pro His Ala Asp Leu Gln Ala Met Ala Arg
    690                 695                 700

gct cat cga ctt ggc caa aca aat aag gtg atg att tat agg ctc ata   2160
```

Pklseq1.app

```
Ala His Arg Leu Gly Gln Thr Asn Lys Val Met Ile Tyr Arg Leu Ile
705                 710                 715                 720

aac cga ggc acc att gaa gaa agg atg atg caa ttg act aaa aag aaa   2208

Asn Arg Gly Thr Ile Glu Glu Arg Met Met Gln Leu Thr Lys Lys Lys
                725                 730                 735

atg gtt cta gag cat ctt gtt gtt ggg aaa ctc aaa aca caa aac att   2256

Met Val Leu Glu His Leu Val Val Gly Lys Leu Lys Thr Gln Asn Ile
            740                 745                 750

aat cag gaa gag tta gat gac atc atc agg tat gga tca aag gag ctt   2304

Asn Gln Glu Glu Leu Asp Asp Ile Ile Arg Tyr Gly Ser Lys Glu Leu
        755                 760                 765

ttt gct agt gaa gat gat gaa gca gga aag tct gga aaa att cat tat   2352

Phe Ala Ser Glu Asp Asp Glu Ala Gly Lys Ser Gly Lys Ile His Tyr
    770                 775                 780

gat gat gcg gct ata gac aaa ttg ctt gat cgt gat ctc gtg gag gca   2400

Asp Asp Ala Ala Ile Asp Lys Leu Leu Asp Arg Asp Leu Val Glu Ala
785                 790                 795                 800

gag gaa gtc tca gtg gat gat gaa gag gag aat gga ttc tta aag gct   2448

Glu Glu Val Ser Val Asp Asp Glu Glu Glu Asn Gly Phe Leu Lys Ala
                805                 810                 815

ttc aag gtg gct aat ttt gaa tat ata gat gaa aat gag gca gca gca   2496

Phe Lys Val Ala Asn Phe Glu Tyr Ile Asp Glu Asn Glu Ala Ala Ala
            820                 825                 830

tta gag gca cag aga gtc gct gct gaa agc aaa tct tca gca ggc aat   2544

Leu Glu Ala Gln Arg Val Ala Ala Glu Ser Lys Ser Ser Ala Gly Asn
        835                 840                 845

tct gat aga gca agt tat tgg gaa gag ttg tta aaa gat aaa ttt gag   2592

Ser Asp Arg Ala Ser Tyr Trp Glu Glu Leu Leu Lys Asp Lys Phe Glu
    850                 855                 860

ctg cac cag gct gag gag ctt aat gct ctt gga aaa agg aag aga agt   2640
```

Pklseq1.app

```
Leu His Gln Ala Glu Glu Leu Asn Ala Leu Gly Lys Arg Lys Arg Ser
865                 870                 875                 880

cgc aag cag ttg gta tcc att gaa gaa gat gat ctt gct ggt ttg gaa   2688

Arg Lys Gln Leu Val Ser Ile Glu Glu Asp Asp Leu Ala Gly Leu Glu
                885                 890                 895

gat gtg agc tct gat gga gat gaa agt tat gaa gct gag tca aca gat   2736

Asp Val Ser Ser Asp Gly Asp Glu Ser Tyr Glu Ala Glu Ser Thr Asp
            900                 905                 910

ggt gaa gca gca gga caa gga gtt cag acg ggt cga cgg ccg tac aga   2784

Gly Glu Ala Ala Gly Gln Gly Val Gln Thr Gly Arg Arg Pro Tyr Arg
        915                 920                 925

aga aag ggt cgc gat aat ttg gaa cca act ccg ttg atg gaa ggt gag   2832

Arg Lys Gly Arg Asp Asn Leu Glu Pro Thr Pro Leu Met Glu Gly Glu
    930                 935                 940

ggg aga tct ttc aga gta ctg ggt ttc aac cag agt caa agg gcc att   2880

Gly Arg Ser Phe Arg Val Leu Gly Phe Asn Gln Ser Gln Arg Ala Ile
945                 950                 955                 960

ttt gta cag act ttg atg agg tat gga gct ggc aat ttt gat tgg aag   2928

Phe Val Gln Thr Leu Met Arg Tyr Gly Ala Gly Asn Phe Asp Trp Lys
                965                 970                 975

gag ttt gtt cct cgc tta aag cag aag acc ttt gaa gaa ata aat gaa   2976

Glu Phe Val Pro Arg Leu Lys Gln Lys Thr Phe Glu Glu Ile Asn Glu
            980                 985                 990

tat gga ata ctc ttc ttg aag cac att gct gaa gaa ata gac gag aat   3024

Tyr Gly Ile Leu Phe Leu Lys His Ile Ala Glu Glu Ile Asp Glu Asn
        995                1000                1005

tct cca acc ttt tca gat ggt gtg ccc aag gaa gga ctt aga ata gaa   3072

Ser Pro Thr Phe Ser Asp Gly Val Pro Lys Glu Gly Leu Arg Ile Glu
    1010                1015                1020

gat gtt cta gtc aga att gct ctt ctg ata cta gtt cag gag aag gtg   3120

Asp Val Leu Val Arg Ile Ala Leu Leu Ile Leu Val Gln Glu Lys Val
```

Pklseq1.app

```
1025                1030                1035                1040

aaa ttt gta gaa gat cat cca ggg aaa cct gtt ttc ccc tct cgc att   3168

Lys Phe Val Glu Asp His Pro Gly Lys Pro Val Phe Pro Ser Arg Ile
                1045                1050                1055

ctt gaa aga ttc ccc gga ctg aga agt gga aaa att tgg aag gag gaa   3216

Leu Glu Arg Phe Pro Gly Leu Arg Ser Gly Lys Ile Trp Lys Glu Glu
            1060                1065                1070

cat gac aag ata atg ata cgt gct gtt tta aag cat ggg tac gga cgg   3264

His Asp Lys Ile Met Ile Arg Ala Val Leu Lys His Gly Tyr Gly Arg
        1075                1080                1085

tgg caa gct att gtt gat gac aaa gag ttg ggg atc caa gag ctt atc   3312

Trp Gln Ala Ile Val Asp Asp Lys Glu Leu Gly Ile Gln Glu Leu Ile
    1090                1095                1100

tgc aaa gaa ttg aat ttc cct cac ata agt ttg tct gct gct gaa caa   3360

Cys Lys Glu Leu Asn Phe Pro His Ile Ser Leu Ser Ala Ala Glu Gln
1105                1110                1115                1120

gct ggt ttg cag ggg cag aat ggt agt ggg ggc tct aat ccg gga gca   3408

Ala Gly Leu Gln Gly Gln Asn Gly Ser Gly Gly Ser Asn Pro Gly Ala
                1125                1130                1135

cag act aac cag aat cct gga agc gtt att act ggg aac aat aat gct   3456

Gln Thr Asn Gln Asn Pro Gly Ser Val Ile Thr Gly Asn Asn Asn Ala
            1140                1145                1150

tct gct gat ggg gct caa gta aac tcg atg ttc tat tat cgg gac atg   3504

Ser Ala Asp Gly Ala Gln Val Asn Ser Met Phe Tyr Tyr Arg Asp Met
        1155                1160                1165

cag aga cga ctt gtt gag ttt gtg aaa aag cga gtt ctg ctt ttg gag   3552

Gln Arg Arg Leu Val Glu Phe Val Lys Lys Arg Val Leu Leu Leu Glu
    1170                1175                1180

aag gcg atg aat tat gaa tac gca gag gaa tat tat gga ctt ggt ggc   3600

Lys Ala Met Asn Tyr Glu Tyr Ala Glu Glu Tyr Tyr Gly Leu Gly Gly
1185                1190                1195                1200
```

Pklseq1.app

```
tca tca tct atc cct act gaa gaa cca gaa gct gaa cca aag atc gct   3648

Ser Ser Ser Ile Pro Thr Glu Glu Pro Glu Ala Glu Pro Lys Ile Ala
                1205                1210                1215

gac aca gtg gga gtg agc ttt att gag gtt gat gat gaa atg ctt gat   3696

Asp Thr Val Gly Val Ser Phe Ile Glu Val Asp Asp Glu Met Leu Asp
            1220                1225                1230

gga ctt cct aag act gat cct atc act tca gaa gaa att atg ggg gct   3744

Gly Leu Pro Lys Thr Asp Pro Ile Thr Ser Glu Glu Ile Met Gly Ala
        1235                1240                1245

gct gtt gac aac aac caa gcg cgg gtc gaa ata gct caa cat tat aac   3792

Ala Val Asp Asn Asn Gln Ala Arg Val Glu Ile Ala Gln His Tyr Asn
    1250                1255                1260

cag atg tgc aaa ctt ctt gat gag aac gct cgg gaa tca gtc caa gca   3840

Gln Met Cys Lys Leu Leu Asp Glu Asn Ala Arg Glu Ser Val Gln Ala
1265                1270                1275                1280

tat gta aac aac caa cca ccg agt acc aag gtg aat gag agc ttc cgt   3888

Tyr Val Asn Asn Gln Pro Pro Ser Thr Lys Val Asn Glu Ser Phe Arg
                1285                1290                1295

gca ctc aaa tct atc aat ggt aac att aac aca atc ctt tcg att aca   3936

Ala Leu Lys Ser Ile Asn Gly Asn Ile Asn Thr Ile Leu Ser Ile Thr
            1300                1305                1310

tct gat caa tcc aag tca cat gaa gac gac acc aag cca gac cta aac   3984

Ser Asp Gln Ser Lys Ser His Glu Asp Asp Thr Lys Pro Asp Leu Asn
        1315                1320                1325

aat gtt gag atg aag gac acg gcc gaa gaa aca aaa ccg tta aga ggt   4032

Asn Val Glu Met Lys Asp Thr Ala Glu Glu Thr Lys Pro Leu Arg Gly
    1330                1335                1340

ggc gtc gtc gat ctg aat gtg gtg gag gga gag gag aac att gct gaa   4080

Gly Val Val Asp Leu Asn Val Val Glu Gly Glu Glu Asn Ile Ala Glu
1345                1350                1355                1360
```

Pklseq1.app

gct agt gga agt gtt gat gta aaa atg gaa gaa gcc aaa gaa gaa gag 4128

Ala Ser Gly Ser Val Asp Val Lys Met Glu Glu Ala Lys Glu Glu Glu
1365 1370 1375

aag cca aag aac atg gtc gtt gat tgactcaact ggtaaatcaa gattc 4177

Lys Pro Lys Asn Met Val Val Asp
1380

<210> 2

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> AFLP Primer EcoRI for AFLP Mapping Analysis in Example 1

<400> 2

agactgcgta ccatttcnn 19

<210> 3

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> AFLP Primer MseI for AFLP Mapping Analysis in Example 1

<400> 3

gatgagtcct gagtaannn 19

<210> 4
<211> 21
<212> DNA
<213> Arabidopsis thaliana
<220>
<221> N/A
<222> sequence complementary to nucleotides 1725-1745 of SEQ ID NO:1
<223> Primers for PCR of Example 2
<400> 4
tgttgagcca gttattcacg a 21

<210> 5
<211> 21
<212> DNA
<213> Arabidopsis thaliana
<220>
<221> N/A
<222> sequence complementary to nucleotides 1934-1914 in SEQ ID NO:1
<223> Primers for PCR of Example 2
<400> 5
acctttccat caattcgctc g 21

<210> 6
<211> 30
<212> DNA

Pklseq1.app

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 2

<400> 6

ccgctcgaga accccaatga ccagctcagt 30

<210> 7

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<222> sequence complementary to nucleotides 672-652 of LEC1 cDNA sequence

<223> Primers for PCR of Example 2

<400> 7

ccttcttcac ttatactgac c 21

<210> 8

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<221>

<222> nucleotides 65-85 of ROC3 cDNA sequence

<223> Primers for PCR of Example 2

Pklseq1.app

<400> 8

aagtctactt cgacatgacc g 21

<210> 9

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<221>

<222> sequence complementary to nucleotides 524-504 of ROC3 cDNA sequence

<223> Primers for PCR of Example 2

<400> 9

cttccagagt cagatccaac c 21

<210> 10

<211> 30

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> N/A

<222> represent nucleotides 895-924 in SEQ ID NO:1 wherein nucleotide 907 is changed from "a" to "g"

<223> Primers for PCR of Example 4

<400> 10

gaaatgggac taggcaggac aattcaaagc 30

Pklseq1.app

<210> 11

<211> 30

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> N/A

<222> represent sequence complementary to nucleotides 924-895 in SEQ ID NO:1, with nucleotide 911 changed from "t" to "c".

<223> Primers for PCR of Example 4

<400> 11

gctttgaatt gtcctgccta gtcccatttc 30

<210> 12

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 4

<400> 12

aagccaaaga acatggtcgt tgatctagag gatcctgaag ctcgaaa 47

<210> 13

<211> 52

<212> DNA

Pklseq1.app

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 4

<400> 13

gaatcttgat ttaccagttg agtcattttt gatgaaacag aagctttttg at 52

<210> 14

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> N/A

<222> represent sequence complementary to nucleotides 4152-4132 in SEQ ID NO:1

<223> Primers for PCR of Example 4

<400> 14

atcaacgacc atgttctttg g 21

<210> 15

<211> 22

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> N/A

<222> represent nucleotides 4153-4174 in SEQ ID NO:1

Pklseq1.app

<223> Primers for PCR of Example 4

<400> 15

tgactcaact ggtaaatcaa ga 22

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 16

ccgctcgagt gagtagtttg gtggagaggc 30

<210> 17

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 17

ccggaattcc atcggaggaa ccttgttcac 30

Pklseq1.app

<210> 18

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 18

cgcggatccc atcggaggaa ccttgttcac 30

<210> 19

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 19

tgctctagat gagtagtttg gtggagaggc 30

<210> 20

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

Pklseq1.app

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 20

ccgctcgagc cctcacataa gtttgtctgc 30

<210> 21

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 21

ccggaattcg tcttaggaag tccatcaagc 30

<210> 22

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primers for PCR of Example 5

<400> 22

Pklseq1.app

cgcggatccg tcttaggaag tccatcaagc 30

<210> 23
<211> 30
<212> DNA
<213> Artificial Sequence
<220>
<221> N/A
<222> N/A
<223> Primers for PCR of Example 5
<400> 23
tgctctagac cctcacataa gtttgtctgc 30

<210> 24
<211> 31
<212> DNA
<213> Artificial Sequence
<220>
<221> N/A
<222> N/A
<223> Primers for PCR of Example 6
<400> 24
cgcggatcct ttttccactt ctcagtccgg g 31

<210> 25
<211> 34
<212> DNA

Pklseq1.app

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Sequence used to form the modified pBluescript vector in Example 4

<400> 25

cttcgaactc gagggatccc catggctagc agct 34

<210> 26

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Sequence used to form the modified pBluescript vector in Example 4

<400> 26

gctagccatg gggatccctc gagttcgaag gtac 34

<210> 27

<211> 12

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

Pklseq1.app

<222> N/A

<223> Primer for forming modified pCAMBIA3300 of Example 4

<400> 27

ccaggtacct gg 12

<210> 28

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Primer for forming modified pCAMBIA3300 of Example 4

<400> 28

aattccaggt acctggcatg 20

<210> 29

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<221> N/A

<222> N/A

<223> Sequence for forming clone of the rat glucocorticoid receptor in Example 4

<400> 29

tctagaggat cctgaagctc gaaaaacaaa gaaaaaaa 38

refers generally to the identity of a tissue during, or at a stage of, development that is brought about by expression of selected genes. For example, selected genes may be expressed in a plant that gives rise to embryonic roots, and thus the developmental identity of the root is embryonic. Furthermore, selected genes may be expressed in a plant that gives rise to seedling roots, and thus the developmental identity of the root is seedling. With specific reference to PKL in pickle roots, one or more genes that gives rise to embryonic roots and one or more genes that gives rise to seedling roots are expressed simultaneously, thus the developmental identity of the root is both embryonic and seedling. The polypeptides described herein are substantially pure (i.e., the proteins are essentially free, e.g., at least about 95% free, from other proteins with which they naturally occur). In one preferred embodiment, the amino acid sequence of a PKL protein having the domains described above, originally found in *Arabidopsis thaliana*, is set forth in SEQ ID:2.

Although the invention is described with reference to *Arabidopsis thaliana* amino acid sequences, it is understood that the invention is not limited to the specific amino acid sequence set forth in SEQ ID:2. Skilled artisans will recognize that, through the process of mutation and/or evolution, polypeptides of different lengths and having differing constituents, e.g., with amino acid insertions, substitutions, deletions, and the like, may arise that are related to, or sufficiently similar to, a sequence set forth herein by virtue of amino acid sequence homology and advantageous functionality as described herein. The term "PKL protein" is used to refer generally to a protein having the features described herein and a preferred example includes a polypeptide having the amino acid sequence of SEQ ID NO:2. Also included within this definition, and in the scope of the invention, are variants of the polypeptide which function in regulating developmental identity, as described herein. Preferred proteins are recombinant proteins.

It is well known that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and yet which effectively provide similar function. For example, an amino acid sequence isolated from another species may differ to a certain degree from the sequence set forth in SEQ ID NO:2, and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although not being limited by theory, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have good activity, even where alterations exist in other portions thereof.

In this regard, a variant of the multi-domain protein described herein, such as a PKL protein variant, is expected to be functionally similar to that set forth in SEQ ID NO:2, for example, if it includes amino acids which are conserved among a variety of species or if it includes non-conserved amino acids which exist at a given location in another species that expresses a functional PKL protein.

Another manner in which similarity may exist between two amino acid sequences is where a given amino acid of one group (such as a non-polar amino acid, an uncharged polar amino acid, a charged polar acidic amino acid or a charged polar basic amino acid) is substituted with another amino acid from the same amino acid group. For example, it is known that the uncharged polar amino acid serine may commonly be substituted with the uncharged polar amino acid threonine in a polypeptide without

substantially altering the functionality of the polypeptide. If one is unsure whether a given substitution will affect the functionality of the enzyme, then this may be determined without undue experimentation using synthetic techniques and screening assays known in the art.

The invention therefore also encompasses amino acid sequences similar to the amino acid sequences set forth herein that have at least about 30% identity thereto and function in regulating developmental identity. Preferably, inventive amino acid sequences have at least about 50% identity, further preferably at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to these sequences.

In preferred embodiments, the invention also encompasses amino acid sequences similar to the amino acid sequences making up polypeptides having the domains described herein. For example, the invention encompasses amino acid sequences that have at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to a first chromo domain from amino acid 115 to amino acid 151 or a second chromo domain extending from amino acid 191 to amino acid 227, at least about 50%, preferably at least about 70%, and more preferably at least about 90% identity to a helicase domain extending from amino acid 293 to amino acid 739, and at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to a DNA binding domain extending from amino acid 1069 to amino acid 1095, and combinations thereof, all as set forth in SEQ ID NO:2. The invention further encompasses amino acid sequences, in addition to those amino acid sequences described above, that have at least about 50%, preferably at least about 70% and more preferably at least about 90% identity to the zinc finger domain amino acid sequence from amino acid 49 to amino acid 96.

Percent identity may be determined, for example, by comparing sequence information using the advanced BLAST computer program, version 2.0, available from the National Institutes of Health. The BLAST

binding domain, or other protein as described herein, such as one having an amino acid sequence having the selected percent identities to the various domains in SEQ ID NO:2 as described herein, including the zinc finger domain. The cell is typically cultured for a time period and under conditions effective for hybridization of the antisense nucleic acid sequence to nucleic acid of the host. The antisense nucleic acid sequence may be DNA or RNA. The length of nucleotides the antisense nucleotide sequence may be complementary to is typically a length sufficient for hybridization to the target nucleic acid sequence so that transcription and/or translation will be substantially inhibited and/or production of a functional protein will be substantially stopped or otherwise substantially decreased. For example, antisense nucleotide sequence may be at least about 25 nucleotides long, and may further be about 50 to about 4200 nucleotides long, preferably about 100 to about 1000 nucleotides long, and further more preferably about 200 to about 500 nucleotides long. In preferred forms of the invention, the antisense nucleic acid sequence may be complementary to, for example, a region from about nucleotide 2 to about nucleotide 331 set forth in SEQ ID NO:1. In other preferred forms of the invention, the antisense nucleic acid sequence may be complementary to a region from about nucleotide 3330 to about nucleotide 3710 in SEQ ID NO:1.

In yet another form of a method of transforming a host cell, a method may include introducing into the host cell a vector that includes a nucleic acid molecule that may be used to generate a nucleic acid molecule, such as an antisense RNA molecule, that will bind to the endogenous transcript in order to inhibit translation of the transcript and to target the transcript for degradation. In one form, the method may include introducing into the host cell a vector that includes length of nucleotides within the nucleotide sequence shown in SEQ ID NO:1 along with the same nucleotides in an antisense orientation. As an example, the host cell may be transformed with a construct that includes, in the following order, a promoter, operably linked to, for example, a PKL fragment as described herein in the sense

Ecker, J. R. (1994) *Genomics* 19:137-144]. The AFLP analysis was performed as described by Liscum, E. and Oeller, P. W. (1999) *Genome Analysis, P. Offner* (Ed.),. CRC Press, Boca Raton, FL, in press]. The AFLP primers used for mapping analysis were as follows: the basic EcoRI primer is 5'-AGA CTG CGT ACC ATT TCx y-3' (where x and y indicate base pairs added for specificity), shown in SEQ ID NO:3, and the basic MseI primer is 5'-GAT GAG TCC TGA GTA Axy z-3' (where x, y, and z indicate base pairs added for specificity), shown in SEQ ID NO:4. E11M48 denotes the primer pair EcoRI-AA and MseICAC, E11M49 denotes the primer pair EcoRI-AA and MseI-CAG, and EI4M59 denotes the primer pair EcoRI-AT and MseICTA [Alonso-Blanco, C. et al. (1998) *Plant J.* 14: 259-271].

To identify polymorphisms in the fast neutron-derived alleles of *PKL*, Southern blots were performed using genomic DNA from plants and digoxigenin-labeled probes that were generated from YAC DNA using AFLP technology. DNA from YAC CIC8H12 (YAC CIC8H12 was obtained from the Arabidopsis Biological Resource Center, Columbus, Ohio) and was prepared as described [Gibson, S. I. and Somerville, C. (1992) *World Scientific:* 119-143]. Approximately 50 ng of CIC8H12 DNA was utilized in a restriction and ligation reaction as described at http://carnegiedpb.stanford.edu/methods/aflp.htmi, with the following differences: the DNA was only digested with MseI, and only the MseI adaptor was ligated on. Five µl of this restriction and ligation (RAL) mixture was then used in a 100 µl digoxigenin-labeling PCR reaction (Roche Biochemicals, cat. # 1 636 090) with 100 pmol each of 6 MseI-xy primers (where x and y indicate base pairs added for specificity). The entire PCR reaction was then used to probe a Southern blot as described in the Dig User's Guide (Roche Biochemicals, cat. # 1 438 425). Random combinations of 6 MseI-xy primers were used to screen for polymorphisms in the fast neutron-derived alleles. Polymorphisms were revealed when the following 6 primers were utilized: xy = CT, GG, GC, AG, TG, AT.

EXAMPLE 2

Characterization of PKL

Ribonuclease protection assays.

Ribonuclease protection assays were performed using the RPA III kit from Ambion (cat. # 1414). To generate a PKL-specific probe, a DNA fragment was generated via RT-PCR using the primers JOpr244 (5'-TGT TGA GCC AGT TAT TCA CGA-3'), (nucleotides 1725-1745 in SEQ ID NO:1) shown in SEQ ID NO:5, and JOpr247 (5'-ACC TTT CCA TCA ATT CGC TCG-3') (sequence complementary to nucleotides 1934-1914 in SEQ ID NO:1) shown in SEQ ID NO:6, and subcloned using the pGEM-T vector system (Promega, cat. # A3600) in an orientation such that the T7 promoter would produce an anti-sense transcript. This plasmid was called pJ0657. To generate a *LEC1*-specific probe, a DNA fragment was generated via PCR using the primers JOpr273 (5'CCGCTCGAGAACCCCAATGACCAGCTCAGT-3'), shown in SEQ ID NO:7 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 33-53 of LEC1 cDNA sequence, Genbank Accession No. AF036684), and JOpr262 (5'-CCTTCTTCACTTATACTGACC-3'), shown in SEQ ID NO:8 (sequence complementary to nucleotides 672-652 of LEC1 cDNA sequence, Genbank Accession No. AF036684), digested with XhoI and KpnI and subcloned into pBluescript SK cut with XhoI and KpnI to produce pJ0660. To generate a ROC3-specific probe, a DNA fragment was generated via PCR using the primers JOpr276 (5'-AAGTCTACTTCGACATGACCG-3'), shown in SEQ ID NO:9 (nucleotides 65-85 of ROC3 cDNA sequence, Genbank Accession No. U40399), and JOpr277 (5'-CTTCCAGAGTCAGATCCAACC-3'), shown in SEQ ID NO:10 (sequence complementary to nucleotides 524-504 of ROC3 cDNA sequence, Genbank Accession No. U40399), and subcloned using the pGEM-T vector system in an orientation such that the T7 promoter would produce an anti-sense transcript. This plasmid was called

In conclusion, cloning of a gene necessary for repression of embryonic identity has lead to the proposition that a GA-modulated chromatin remodeling factor mediates a developmental transition in *Arabidopsis*. It is anticipated that further characterization of *PKL*, and identification of proteins that either regulate or are targets of *PKL*, will shed light on the mechanism of GA signal transduction and the role of GA in regulating differentiation and development in *Arabidopsis*. It remains to be determined whether CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events.

EXAMPLE 4

Generation of Mutant PKL by a Dominant Negative Strategy

It has previously been demonstrated that a point mutation of a conserved lysine in the ATPase/helicase domain of SWI/SNF proteins generates a dominant negative mutant form of the protein [Chavari et al., (1993) *Nature* 366:170-174). By mutating the analogous mutation in PKL (by mutating Lys-304 to an Arg residue), a dominant negative version of PKL may be generated. This mutant allele of *PKL* may be generated by a PCR strategy.

A complementation construct for PKL was generated that includes the PKL cDNA flanked by 1.1 kb of upstream genomic sequence (to the BstBI site) and 1.4 kb of downstream genomic sequence (to the NcoI site). The construct was generated by performing overlap PCR on PKL cDNA with three DNA fragments: the genomic fragment upstream of the PKL start codon to the BstBI site, the PKL cDNA and the genomic fragment downstream of the termination codon to the NcoI site. A BstBI – XhoI fragment (2.1 kb) from this construct has been subcloned into a modified pBluescript vector (pJO674). The modified pBluescript vector pJ0674 was formed by ligating in a cassette generated by annealing the primers JOpr386 (5'-CTTCGAACTCGAGGGATCCCCATGGCTAGCAGCT-3'), shown in SEQ ID NO:26 (this is a synthetic sequence that includes "A"

followed by the recognition sequence of BstB1, Xhol, Bam HI, Ncol, Nhe I and sequence "AGCT" wherein the last "G" in the Ncol recognition sequence and the first "G" in the Nhel recognition sequence overlap) and JOpr387(5'-GCTAGCCATGGGGATCCCTCGAGTTCGAAGGTAC), as shown in SEQ ID NO:27 (this is a synthetic sequence complementary to SEQ ID NO:26) after pBluescript was cut with Kpnl and Sacl. The resulting cassette include the following restriction sites: BstB1, Xhol, Bam HI, Ncol and Nhel. 2 separate PCR reactions have been performed using this vector as a substrate. 1 PCR reaction uses a T3 primer with the following primer shown in SEQ ID NO:11 (JOpr516) 5'-GAAATGGGACTAGGCAGGACAATTCAAAGC-3' (nucleotides 895-924 in SEQ ID NO:1) where the underlined G is designed to replace an A residue in the wild-type PKL sequence and introduce the Lys-304 to Arg-304 mutation. This reaction generates a 272 bp fragment. The other PCR reaction uses a T7 primer with the following primer shown in SEQ ID NO:12 (JOpr517) 5'-GCTTTGAATTGTCCTGCCTAGTCCCATTTC-3' (sequence complementary to SEQ ID NO:1 from nucleotides 924-895) where the underlined C is designed to replace a T residue in the wild-type PKL sequence and introduce the Lys-304 to Arg-304 mutation. This reaction generates a 2094 bp fragment. Overlap PCR can then be done by adding the 272 bp and 2094 bp fragment together along with the T3 and T7 primers generating a 2.3 kb fragment. This fragment will be digested with BstBI and Xhol, cloned back into pJO674 and then sequenced to verify introduction of the mutation. This vector will then be cut with BstBI and Xhol and ligated into a pBluescript-based vector carrying the complementation construct (pJO765, formed by ligating the complementation fragment into pJO674 cut with BstBI and Ncol) cut with BstBI and Xhol, resulting in generation of a complementation construct that carries the dominant negative mutation. This construct will then be transferred to a binary vector [a modified pCAMBIA3300, pJO630, which is formed by digesting pCAMBIA3300 with BstXI and EcoRI and ligating in the

cassette generated by annealing primers JOpr232 (5'-CCAGGTACCTGG-3'), shown in SEQ ID NO:28 and JOpr233 (5'-AATTCCAGGTACCTGGCATG-3'), shown in SEQ ID NO:29] and transformed into wild-type plants to verify generation of a mutant *pkl* phenotype. These sequences are synthetic sequences that anneal to form a cassette that has ends that are compatible to BstXI and EcoRI digested pCAMBIA3300. The entire sequence of JOpr232 is a new site that when cut with BstXI generates ends that are compatible with KpnI ends. The cassette thus recreates a BstXI site with KpnI compatible ends. The PCR reactions and subcloning are performed as known in the art, and as described, for example, in Sambrook et al. (Eds.), *Molecular Cloning: A Laboratory Manua*l, 2nd ed., Cold Spring Harbor Laboratory Press (1989).

A conditional version of this dominant negative allele may be made by fusing the gene to the glucocorticoid receptor [Lloyd et al., (1994) *Science* 266:436-439). A clone of the rat glucocorticoid receptor (GR) was obtained from Alan Lloyd, at the University of Texas, Austin, Texas. The clone included SEQ ID NO:30 (5'-TCTAGAGGATCCTGAAGCTCGAAAAACAAAGAAAAAAA-3'), that is fused to nucleotides 1569-2407 of rat glucocorticoid receptor cDNA found in Genbank Accession No. Y12264. SEQ ID NO:30 was used to add spacers and restriction sites to the clone. A PCR reaction has been performed with this GR clone as a substrate and the following primers: JOpr533 (5'-AAGCCAAAGAACATGGTCGTTGATCTAGAGGATCCTGAAGCTCGAAA-3') shown in SEQ ID NO:13 (the first 24 nucleotides are nucleotides 4129-4152 of SEQ ID NO:1 whereas the last 23 nucleotides are nucleotides 2-24 of SEQ ID NO:30 of the rat glucocorticoid receptor cDNA found in Genbank Accession No. Y12264) and JOpr534 (5'-GAATCTTGATTTACCAGTTGAGTCATTTTTGATGAAACAGAAGCTTTTT GAT-3') (the first 25 nucleotides are nucleotides complementary to nucleotides 4153-4177 of SEQ ID NO:1 and the last 27 nucleotides are

complementary to nucleotides 2407-2381 of the glucocorticoid receptor cDNA found in Genbank Accession No. Y12264) shown in SEQ ID NO:14, which are designed to add PKL sequences to the end of the GR fragment such that overlap PCR can be performed. A BamHI –NcoI fragment of the complementation construct has been subcloned into pJO674, generating vector pJO724. pJO724 may be the substrate for 2 PCR reactions. One reaction can use the T7 primer and JOpr398 (5'-ATCAACGACCATGTTCTTTGG-3') (sequence complementary to nucleotides 4152-4132 of SEQ ID NO:1), shown in SEQ ID NO:15, generating a 883 bp fragment. The other reaction will use the T3 primer and JOpr401 (5'- TGACTCAACTGGTAAATCAAGA-3') (nucleotides 4153-4174 of SEQ ID NO:1), shown in SEQ ID NO:16, generating a 1.5 kb fragment. Overlap PCR can then be performed using 883 bp fragment and the GR fragment with the T7 primer and JOpr534. Overlap PCR can then be performed again using the product of this PCR reaction and the 1.5 kb fragment using the T7 primer and the T3 primer. This PCR product can then be digested with BamHI and NcoI and cloned back into pJO674 digested with the same. The construct will then be sequenced to verify identity. This construct can then be digested with BamHI and NcoI and ligated to the dominant-negative version of the complementation construct to generate a C-terminal fusion of GR to the mutant PKL protein. Once again, this construct can be transferred to a binary vector (pJO630) and transformed into wild-type plants to verify that a mutant *pkl* phenotype will be generated upon addition of dexamethasone.

If necessary, the dominant-negative version of the gene may be overexpressed in order to generate a phenotype. In this case, the mutated ORF (+/- GR) can be cloned downstream of a constitutive high level promoter such as the 35S promoter in a binary vector.

In all of Examples 4-6 described herein, ribonuclease protection assays will be performed to verify expression of the mutant transcript. The

pkl phenotype will be assayed by penetrance of the pickle root phenotype and by the rosette phenotype [Ogas, J. et al. (1997) *Science* 277:91-94].

EXAMPLE 5

Generation of Mutant PKL by Antisense Procedures

Two constructs for inhibiting expression of endogenous *PKL* by iRNA may be generated. These constructs are based on sequence comparison between PKL and PKR2, which is another CHD protein that exhibits high sequence similarity to PKL. A fragment of PKL may be cloned into the vector pRNA69, which results in formation of the following construct: 35S promoter – *PKL* frag in sense orientation – intron – the same PKL frag in antisense orientation – terminator. Vector pRNA69 is a bacterial vector that was obtained from John Bowman at UC Davis.

The sequence of the *PKL* cDNA that is being targeted in the first construct is from nucleotide 2 to nucleotide 361 in SEQ ID NO:1. This fragment was generated by performing PCR on PKL cDNA with the following primers: JOpr442 (5'-CCGCTCGAGTGAGTAGTTTGGTGGAGAGGC-3') found in SEQ ID NO:17 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 2-22 of SEQ ID NO:1) and JOpr443 (5'-CCGGAATTCCATCGGAGGAACCTTGTTCAC-3'), found in SEQ ID NO:18 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Eco RI recognition sequence whereas the last 21 nucleotides are complementary to nucleotides 361-341 of SEQ ID NO:1), for the cloning the sense orientation (as a XhoI-EcoRI fragment) and JOpr444 (5'-CGCGGATCCCATCGGAGGAACCTTGTTCAC-3'), shown in SEQ ID NO:19 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI

recognition sequence and the last 21 nucleotides are complementary to nucleotides 361-341 of SEQ ID NO:1) and JOpr445 (5'-TGCTCTAGATGAGTAGTTTGGTGGAGAGGC-3'), shown in SEQ ID NO:20 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XbaI recognition sequence and the last 21 nucleotides are nucleotides 2-22 of SEQ ID NO:1), for cloning the antisense orientation (as a BamHI-XbaI fragment) into pRNA69.

The sequence of the *PKL* cDNA that is being targeted in the second construct is from nucleotide 3330 to nucleotide 3710 in SEQ ID NO:1. This fragment was generated by performing PCR on PKL cDNA with the following primers: JOpr446 (5'-CCGCTCGAGCCCTCACATAAGTTTGTCTGC-3'), shown in SEQ ID NO:21 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XhoI recognition sequence and the last 21 nucleotides are nucleotides 3330-3349 of SEQ ID NO:1), and JOpr447 (5'-CCGGAATTCGTCTTAGGAAGTCCATCAAGC-3'), shown in SEQ ID NO:22 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Eco RI recognition sequence and the last 21 nucleotides are complementary to nucleotides 3710-3690 of SEQ ID NO:1), for the cloning the sense orientation (as a XhoI-EcoRI fragment) and JOpr448 (5'-CGCGGATCCGTCTTAGGAAGTCCATCAAGC-3'), found in SEQ ID NO:23 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI recognition sequence whereas the last 21 bases are nucleotides 3330-3351 in SEQ ID NO:1), and JOpr449 (5'-TGCTCTAGACCCTCACATAAGTTTGTCTGC-3'), shown in SEQ ID NO:24 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the XbaI recognition

sequence and the last 21 nucleotides are nucleotides 3330-3350 in SEQ ID NO:1)for cloning the antisense orientation (as a BamHI-XbaI fragment) into pRNA69.

The pRNA69 constructs may then be ligated into the binary vector pBART by making use of the flanking NotI sites. Wild-type plants may then be transformed by these constructs by vacuum infiltration. The plants may then be screened for a mutant pkl phenotype as described for Example 5.

EXAMPLE 6

Generation of Mutant PKL by Domain Deletion

It has been shown that removing the DNA-binding portion of *CHD1* in *S. cerevisiae* generates an inactive form of the protein [Woodage et al., (1997) *PNAS* 94:11472-11477). By specifically deleting the DNA-binding domain (aa 1069 - 1095) or any of the other domains, a dominant negative version of *PKL* may be produced. The XhoI-BamHI fragment of the PKL cDNA sequence has been cloned into pJO687, a vector obtained by introducing this fragment into a pJO674 vector formed as described in Example 4. In order to delete the putative DNA binding domain of PKL, PCR mutagenesis may be used. Briefly, a PCR reaction may be performed using pJO687 as a substrate and T7 and the oligo 5'-CGCGGATCCTTTTTCCACTTCTCAGTCCGGG-3', shown in SEQ ID NO:25 (the first 3 nucleotides are used as spacers so the restriction enzyme will cut properly, the next 6 nucleotides represent the Bam HI recognition sequence and the last 21 nucleotides are complementary to nucleotides 3202-3181 of SEQ ID NO:1), as a primer. The product can be digested with XhoI and BamHI and cloned into pJO674 cut with the same, and then can be sequenced to verify introduction of the mutation. This vector can then be cut with XhoI and BamHI and ligated into a pBluescript-based vector, carrying the complementation construct (pJO765) cut with the same, resulting in generation of a complementation construct that carries PKL deleted for the DNA binding domain. This construct can then

6. The method of claim 3, wherein said second chromo domain is encoded by a nucleic acid molecule having a nucleotide sequence having at least about 50% identity to the nucleotide sequence set forth in SEQ ID NO:1 from nucleotide 571 to nucleotide 681.

7. The method of claim 1, wherein said nucleic acid molecule has a nucleotide sequence encoding protein domains selected from the group consisting of a chromo domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 from amino acid 115 to amino acid 151, a helicase domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 from amino acid 293 to amino acid 739 and a DNA binding domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 from amino acid 1069 to amino acid 1095.

8. The method of claim 2, wherein said nucleic acid molecule has a nucleotide sequence encoding said zinc finger domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 from amino acid 49 to amino acid 96.

9. The method of claim 3, wherein said nucleic acid molecule has a nucleotide sequence encoding said second chromo domain having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 from amino acid 191 to amino acid 227.

10. The method of claim 1, wherein said host cell is a eukaryotic cell.

11. The method of claim 10, wherein said eukaryotic cell is a plant cell.

12. The method of claim 11, wherein said eukaryotic cell is an animal cell.

13. The method of claim 12, wherein said animal cell is a mammalian cell.

14. The method of claim 13, wherein said mammalian cell is a human cell.

15. The method of claim 1, further comprising deleting the nucleotide sequences encoding any one of said domains prior to said introducing.

16. The method of claim 1, wherein said protein has a point mutation in lysine 304 of SEQ ID NO:2.

17. The method of claim 16, wherein said mutation results in said lysine being replaced by an arginine.

18. The method of claim 1, wherein said protein encodes PKL.

19. The method of claim 18, wherein said PKL has an amino acid sequence as set forth in SEQ ID NO:2.

20. The method of claim 1, wherein said nucleic acid molecule has a nucleotide sequence having at least about 80% identity to the nucleotide sequence set forth in SEQ ID NO:1.

21. The method of claim 1, wherein said nucleic acid molecule further comprises a promoter operably linked to a terminal 5' end of said nucleotide sequence.

22. The method of claim 21, wherein said promoter is selected from the group consisting of a constitutive promoter, an inducible promoter and a cell-specific promoter.

23. The method of claim 21, wherein said promoter is a foreign promoter.

24. The method of claim 18, wherein said PKL functions in repressing embryonic identity in said plant.

25. The method of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1.

26. A method of transforming a host cell, comprising introducing into a host cell a nucleic acid molecule encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2, said protein functioning in regulating developmental identity.

27. The method of claim 26, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:2.

28. The method of claim 27, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:2.

51. The method of claim 45, wherein said nucleotide sequence is complementary to a region from about nucleotide 3330 to about nucleotide 3710 set forth in SEQ ID NO:1.

52. A method of transforming a host cell, comprising:

(a) introducing into a host cell a vector comprising a first nucleic acid molecule having a nucleotide sequence that is complementary to a nucleotide sequence having at least about 50% identity to a length of nucleotides within the nucleotide sequence set forth in SEQ ID NO:1, said nucleotide sequence encoding a protein functioning in regulating developmental identity;

(b) generating an antisense nucleic acid molecule complementary to an RNA transcript formed from SEQ ID NO:1; and

(b) culturing said host cell under conditions effective for hybridization of said antisense molecule to said RNA transcript of said host cell.

53. The method of claim 52, wherein said nucleic acid molecule has a nucleotide sequence that is complementary to a length of nucleotides within the nucleotide sequence set forth in SEQ ID NO:1.

54. The method of claim 52, wherein the antisense nucleic acid molecule is an RNA molecule.

55. A recombinant nucleic acid molecule, comprising:

(a) a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having at least one chromo domain, a helicase domain and a DNA binding domain, said protein expressible in an amount sufficient to regulate developmental identity; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

56. The method of claim 55, wherein said protein further has at least one zinc finger domain.

57. The method of claim 55, wherein said protein further has a second chromo domain.

58. A recombinant nucleic acid molecule, comprising:

(a) a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2 ; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

59. The molecule of claim 58, wherein said foreign promoter is selected from the group consisting of a constitutive promoter, an inducible promoter and a cell-specific promoter.

60. The molecule of claim 58, wherein said protein has an amino acid sequence having at least about 70% identity to the amino acid sequence set forth in SEQ ID NO:2.

61. The molecule of claim 58, wherein said protein has an amino acid sequence of PKL.

62. The molecule of claim 61, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:2.

70. A eukaryotic cell, comprising:

(a) an introduced nucleic acid molecule having a nucleotide sequence encoding a protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2.

71. The cell of claim 70, wherein said protein has an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2.

72. The cell of claim 71, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:2.

73. The cell of claim 70, wherein said cell is a plant cell.

74. The cell of claim 70, wherein said cell is an animal cell.

75. A transgenic plant, comprising:

(a) an introduced nucleic acid molecule having a nucleotide sequence encoding a plant protein functioning in regulating developmental identity, said protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2; and

(b) a foreign promoter operably linked to a terminal 5' end of said nucleotide sequence.

76. The transgenic plant of claim 75, wherein said nucleotide sequence is an antisense DNA or RNA molecule.

77. The transgenic plant of claim 75, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:2.

78. The transgenic plant of claim 77, wherein said protein has the amino acid sequence of PKL.

79. The transgenic plant of claim 78, wherein said amino acid sequence is as set forth in SEQ ID NO:2.

80. A recombinant protein, comprising a protein having an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2.

81. The protein of claim 80, wherein said protein has an amino acid sequence having at least about 80% identity to the amino acid sequence set forth in SEQ ID NO:2.

82. The protein of claim 81, wherein said protein has an amino acid sequence as set forth in SEQ ID NO:2.

83. A method of producing a PKL protein, comprising:

(a) introducing a nucleotide sequence encoding a protein having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:2; and

(b) culturing said host cell under conditions effective to achieve expression of the PKL polypeptide.

Pklseq1.app
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Somerville, Christopher R.

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Lys Gln Tyr Leu Val Lys Trp Lys Gly Leu Ser Tyr Leu His Cys Ser
    130                 135                 140

tgg gtg cct gag aag gag ttc cag aag gct tat aag tca aat cat cgt   480

Trp Val Pro Glu Lys Glu Phe Gln Lys Ala Tyr Lys Ser Asn His Arg
145                 150                 155                 160

tta aaa acc aga gtg aac aat ttt cac cgt caa atg gag tca ttc aat   528

Leu Lys Thr Arg Val Asn Asn Phe His Arg Gln Met Glu Ser Phe Asn
                165                 170                 175

aac agc gaa gat gat ttt gtt gcc ata cgt cct gag tgg acc act gtt   576

Asn Ser Glu Asp Asp Phe Val Ala Ile Arg Pro Glu Trp Thr Thr Val
            180                 185                 190

gat cgg att ctt gcc tgc aga gag gaa gat ggg gag ctg gaa tat ctt   624

Asp Arg Ile Leu Ala Cys Arg Glu Glu Asp Gly Glu Leu Glu Tyr Leu
        195                 200                 205

gtc aaa tat aaa gag cta tcc tat gat gaa tgt tat tgg gag tca gaa   672

Val Lys Tyr Lys Glu Leu Ser Tyr Asp Glu Cys Tyr Trp Glu Ser Glu
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210 215 220

tca gac atc tca acc ttc cag aat gaa att caa agg ttc aag gat gta 720

Ser Asp Ile Ser Thr Phe Gln Asn Glu Ile Gln Arg Phe Lys Asp Val
225 230 235 240

aat tct aga act cgc aga agt aaa gat gtt gac cat aaa aga aat ccc 768

Asn Ser Arg Thr Arg Arg Ser Lys Asp Val Asp His Lys Arg Asn Pro
245 250 255

aga gac ttt caa cag ttt gat cat act cct gaa ttc ctc aaa ggc ttg 816

Arg Asp Phe Gln Gln Phe Asp His Thr Pro Glu Phe Leu Lys Gly Leu
260 265 270

tta cat cca tac cag ctt gag gga ctt aat ttt ttg cgg ttc tcg tgg 864

Leu His Pro Tyr Gln Leu Glu Gly Leu Asn Phe Leu Arg Phe Ser Trp
275 280 285

tca aaa cag acg cat gta atc ctt gct gat gaa atg gga cta ggc aag 912

Ser Lys Gln Thr His Val Ile Leu Ala Asp Glu Met Gly Leu Gly Lys
290 295 300

aca att caa agc att gcc ctt tta gct tca ctt ttt gag gag aac ctc 960

Thr Ile Gln Ser Ile Ala Leu Leu Ala Ser Leu Phe Glu Glu Asn Leu
305 310 315 320

att ccg cat ttg gta att gct cct cta tcg act ctg cgt aac tgg gag 1008

Ile Pro His Leu Val Ile Ala Pro Leu Ser Thr Leu Arg Asn Trp Glu
325 330 335

aga gag ttt gcc aca tgg gcc cca cag atg aac gtg gtt atg tat ttt 1056

Arg Glu Phe Ala Thr Trp Ala Pro Gln Met Asn Val Val Met Tyr Phe
340 345 350

ggc act gcg caa gct cga gca gtt atc aga gaa cat gag ttt tac tta 1104

Gly Thr Ala Gln Ala Arg Ala Val Ile Arg Glu His Glu Phe Tyr Leu
355 360 365

tcg aaa gat caa aaa aag atc aag aaa aag aaa tct gga caa ata agt 1152

Ser Lys Asp Gln Lys Lys Ile Lys Lys Lys Lys Ser Gly Gln Ile Ser
370 375 380

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agc gaa agc aag caa aaa aga atc aag ttt gat gtc ctc ctc aca tcg   1200

Ser Glu Ser Lys Gln Lys Arg Ile Lys Phe Asp Val Leu Leu Thr Ser
385                 390                 395                 400

tat gag atg atc aac cta gat tca gca gtt cta aaa cca att aag tgg   1248

Tyr Glu Met Ile Asn Leu Asp Ser Ala Val Leu Lys Pro Ile Lys Trp
                405                 410                 415

gag tgc atg att gtt gat gaa ggt cat cga ctg aaa aat aag gat tca   1296

Glu Cys Met Ile Val Asp Glu Gly His Arg Leu Lys Asn Lys Asp Ser
            420                 425                 430

aag ctg ttc tct tca ttg aca cag tat tca agt aac cac cgt att ctt   1344

Lys Leu Phe Ser Ser Leu Thr Gln Tyr Ser Ser Asn His Arg Ile Leu
        435                 440                 445

ctg aca gga aca cca ctt cag aac aac ttg gat gaa ctt ttc atg ctc   1392

Leu Thr Gly Thr Pro Leu Gln Asn Asn Leu Asp Glu Leu Phe Met Leu
    450                 455                 460

atg cat ttt ctt gat gcg ggg aag ttt gga agt ttg gag gag ttc cag   1440

Met His Phe Leu Asp Ala Gly Lys Phe Gly Ser Leu Glu Glu Phe Gln
465                 470                 475                 480

gag gag ttc aaa gat att aat caa gag gag cag atc tca agg ttg cac   1488

Glu Glu Phe Lys Asp Ile Asn Gln Glu Glu Gln Ile Ser Arg Leu His
                485                 490                 495

aaa atg ttg gct cca cat ttg ctc aga agg gta aaa aaa gac gta atg   1536

Lys Met Leu Ala Pro His Leu Leu Arg Arg Val Lys Lys Asp Val Met
            500                 505                 510

aaa gac atg ccc ccc aaa aag gag ctc att ttg cgt gtt gat ctg agc   1584

Lys Asp Met Pro Pro Lys Lys Glu Leu Ile Leu Arg Val Asp Leu Ser
        515                 520                 525

agt ctg cag aaa gaa tat tac aaa gct att ttt acc cgt aat tat caa   1632

Ser Leu Gln Lys Glu Tyr Tyr Lys Ala Ile Phe Thr Arg Asn Tyr Gln
    530                 535                 540
```

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gta ttg aca aaa aag gga ggt gct caa att tcc ctt aat aac att atg   1680

Val Leu Thr Lys Lys Gly Gly Ala Gln Ile Ser Leu Asn Asn Ile Met
545                 550                 555                 560

atg gaa tta cga aaa gta tgc tgc cat cct tat atg cta gag ggt gtt   1728

Met Glu Leu Arg Lys Val Cys Cys His Pro Tyr Met Leu Glu Gly Val
                565                 570                 575

gag cca gtt att cac gac gca aat gaa gct ttc aaa caa ctt ttg gag   1776

Glu Pro Val Ile His Asp Ala Asn Glu Ala Phe Lys Gln Leu Leu Glu
            580                 585                 590

tct tgt gga aag ctg caa ctt cta gat aaa atg atg gtc aaa ctg aaa   1824

Ser Cys Gly Lys Leu Gln Leu Leu Asp Lys Met Met Val Lys Leu Lys
        595                 600                 605

gag caa gga cac aga gtc cta ata tac aca cag ttt cag cat atg ctg   1872

Glu Gln Gly His Arg Val Leu Ile Tyr Thr Gln Phe Gln His Met Leu
    610                 615                 620

gac tta ctt gaa gac tac tgt acc cat aag aaa tgg cag tac gag cga   1920

Asp Leu Leu Glu Asp Tyr Cys Thr His Lys Lys Trp Gln Tyr Glu Arg
625                 630                 635                 640

att gat gga aag gtt ggc gga gct gag cgg caa ata cgc ata gat cgg   1968

Ile Asp Gly Lys Val Gly Gly Ala Glu Arg Gln Ile Arg Ile Asp Arg
                645                 650                 655

ttc aat gcc aaa aat tct aac aag ttt tgt ttt ttg ctc tcc aca aga   2016

Phe Asn Ala Lys Asn Ser Asn Lys Phe Cys Phe Leu Leu Ser Thr Arg
            660                 665                 670

gct ggt ggc tta gga ata aat ctt gca acg gct gat aca gta atc att   2064

Ala Gly Gly Leu Gly Ile Asn Leu Ala Thr Ala Asp Thr Val Ile Ile
        675                 680                 685

tat gac agt gac tgg aat cct cat gct gat ctt caa gca atg gct aga   2112

Tyr Asp Ser Asp Trp Asn Pro His Ala Asp Leu Gln Ala Met Ala Arg
    690                 695                 700

gct cat cga ctt ggc caa aca aat aag gtg atg att tat agg ctc ata   2160
```

Ala His Arg Leu Gly Gln Thr Asn Lys Val Met Ile Tyr Arg Leu Ile
705 710 715 720

aac cga ggc acc att gaa gaa agg atg atg caa ttg act aaa aag aaa 2208

Asn Arg Gly Thr Ile Glu Glu Arg Met Met Gln Leu Thr Lys Lys Lys
725 730 735

atg gtt cta gag cat ctt gtt gtt ggg aaa ctc aaa aca caa aac att 2256

Met Val Leu Glu His Leu Val Val Gly Lys Leu Lys Thr Gln Asn Ile
740 745 750

aat cag gaa gag tta gat gac atc atc agg tat gga tca aag gag ctt 2304

Asn Gln Glu Glu Leu Asp Asp Ile Ile Arg Tyr Gly Ser Lys Glu Leu
755 760 765

ttt gct agt gaa gat gat gaa gca gga aag tct gga aaa att cat tat 2352

Phe Ala Ser Glu Asp Asp Glu Ala Gly Lys Ser Gly Lys Ile His Tyr
770 775 780

gat gat gcg gct ata gac aaa ttg ctt gat cgt gat ctc gtg gag gca 2400

Asp Asp Ala Ala Ile Asp Lys Leu Leu Asp Arg Asp Leu Val Glu Ala
785 790 795 800

gag gaa gtc tca gtg gat gat gaa gag gag aat gga ttc tta aag gct 2448

Glu Glu Val Ser Val Asp Asp Glu Glu Glu Asn Gly Phe Leu Lys Ala
805 810 815

ttc aag gtg gct aat ttt gaa tat ata gat gaa aat gag gca gca gca 2496

Phe Lys Val Ala Asn Phe Glu Tyr Ile Asp Glu Asn Glu Ala Ala Ala
820 825 830

tta gag gca cag aga gtc gct gct gaa agc aaa tct tca gca ggc aat 2544

Leu Glu Ala Gln Arg Val Ala Ala Glu Ser Lys Ser Ser Ala Gly Asn
835 840 845

tct gat aga gca agt tat tgg gaa gag ttg tta aaa gat aaa ttt gag 2592

Ser Asp Arg Ala Ser Tyr Trp Glu Glu Leu Leu Lys Asp Lys Phe Glu
850 855 860

ctg cac cag gct gag gag ctt aat gct ctt gga aaa agg aag aga agt 2640

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Leu His Gln Ala Glu Glu Leu Asn Ala Leu Gly Lys Arg Lys Arg Ser
865                 870                 875                 880

cgc aag cag ttg gta tcc att gaa gaa gat gat ctt gct ggt ttg gaa   2688

Arg Lys Gln Leu Val Ser Ile Glu Glu Asp Asp Leu Ala Gly Leu Glu
                885                 890                 895

gat gtg agc tct gat gga gat gaa agt tat gaa gct gag tca aca gat   2736

Asp Val Ser Ser Asp Gly Asp Glu Ser Tyr Glu Ala Glu Ser Thr Asp
            900                 905                 910

ggt gaa gca gca gga caa gga gtt cag acg ggt cga cgg ccg tac aga   2784

Gly Glu Ala Ala Gly Gln Gly Val Gln Thr Gly Arg Arg Pro Tyr Arg
        915                 920                 925

aga aag ggt cgc gat aat ttg gaa cca act ccg ttg atg gaa ggt gag   2832

Arg Lys Gly Arg Asp Asn Leu Glu Pro Thr Pro Leu Met Glu Gly Glu
    930                 935                 940

ggg aga tct ttc aga gta ctg ggt ttc aac cag agt caa agg gcc att   2880

Gly Arg Ser Phe Arg Val Leu Gly Phe Asn Gln Ser Gln Arg Ala Ile
945                 950                 955                 960

ttt gta cag act ttg atg agg tat gga gct ggc aat ttt gat tgg aag   2928

Phe Val Gln Thr Leu Met Arg Tyr Gly Ala Gly Asn Phe Asp Trp Lys
                965                 970                 975

gag ttt gtt cct cgc tta aag cag aag acc ttt gaa gaa ata aat gaa   2976

Glu Phe Val Pro Arg Leu Lys Gln Lys Thr Phe Glu Glu Ile Asn Glu
            980                 985                 990

tat gga ata ctc ttc ttg aag cac att gct gaa gaa ata gac gag aat   3024

Tyr Gly Ile Leu Phe Leu Lys His Ile Ala Glu Glu Ile Asp Glu Asn
        995                1000                1005

tct cca acc ttt tca gat ggt gtg ccc aag gaa gga ctt aga ata gaa   3072

Ser Pro Thr Phe Ser Asp Gly Val Pro Lys Glu Gly Leu Arg Ile Glu
   1010                1015                1020

gat gtt cta gtc aga att gct ctt ctg ata cta gtt cag gag aag gtg   3120

Asp Val Leu Val Arg Ile Ala Leu Leu Ile Leu Val Gln Glu Lys Val
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1025                1030                1035                1040

aaa ttt gta gaa gat cat cca ggg aaa cct gtt ttc ccc tct cgc att   3168

Lys Phe Val Glu Asp His Pro Gly Lys Pro Val Phe Pro Ser Arg Ile
                1045                1050                1055

ctt gaa aga ttc ccc gga ctg aga agt gga aaa att tgg aag gag gaa   3216

Leu Glu Arg Phe Pro Gly Leu Arg Ser Gly Lys Ile Trp Lys Glu Glu
            1060                1065                1070

cat gac aag ata atg ata cgt gct gtt tta aag cat ggg tac gga cgg   3264

His Asp Lys Ile Met Ile Arg Ala Val Leu Lys His Gly Tyr Gly Arg
        1075                1080                1085

tgg caa gct att gtt gat gac aaa gag ttg ggg atc caa gag ctt atc   3312

Trp Gln Ala Ile Val Asp Asp Lys Glu Leu Gly Ile Gln Glu Leu Ile
    1090                1095                1100

tgc aaa gaa ttg aat ttc cct cac ata agt ttg tct gct gct gaa caa   3360

Cys Lys Glu Leu Asn Phe Pro His Ile Ser Leu Ser Ala Ala Glu Gln
1105                1110                1115                1120

gct ggt ttg cag ggg cag aat ggt agt ggg ggc tct aat ccg gga gca   3408

Ala Gly Leu Gln Gly Gln Asn Gly Ser Gly Gly Ser Asn Pro Gly Ala
                1125                1130                1135

cag act aac cag aat cct gga agc gtt att act ggg aac aat aat gct   3456

Gln Thr Asn Gln Asn Pro Gly Ser Val Ile Thr Gly Asn Asn Asn Ala
            1140                1145                1150

tct gct gat ggg gct caa gta aac tcg atg ttc tat tat cgg gac atg   3504

Ser Ala Asp Gly Ala Gln Val Asn Ser Met Phe Tyr Tyr Arg Asp Met
        1155                1160                1165

cag aga cga ctt gtt gag ttt gtg aaa aag cga gtt ctg ctt ttg gag   3552

Gln Arg Arg Leu Val Glu Phe Val Lys Lys Arg Val Leu Leu Leu Glu
    1170                1175                1180

aag gcg atg aat tat gaa tac gca gag gaa tat tat gga ctt ggt ggc   3600

Lys Ala Met Asn Tyr Glu Tyr Ala Glu Glu Tyr Tyr Gly Leu Gly Gly
1185                1190                1195                1200
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tca tca tct atc cct act gaa gaa cca gaa gct gaa cca aag atc gct   3648

Ser Ser Ser Ile Pro Thr Glu Glu Pro Glu Ala Glu Pro Lys Ile Ala
                1205                1210                1215

gac aca gtg gga gtg agc ttt att gag gtt gat gat gaa atg ctt gat   3696

Asp Thr Val Gly Val Ser Phe Ile Glu Val Asp Asp Glu Met Leu Asp
            1220                1225                1230

gga ctt cct aag act gat cct atc act tca gaa gaa att atg ggg gct   3744

Gly Leu Pro Lys Thr Asp Pro Ile Thr Ser Glu Glu Ile Met Gly Ala
        1235                1240                1245

gct gtt gac aac aac caa gcg cgg gtc gaa ata gct caa cat tat aac   3792

Ala Val Asp Asn Asn Gln Ala Arg Val Glu Ile Ala Gln His Tyr Asn
    1250                1255                1260

cag atg tgc aaa ctt ctt gat gag aac gct cgg gaa tca gtc caa gca   3840

Gln Met Cys Lys Leu Leu Asp Glu Asn Ala Arg Glu Ser Val Gln Ala
1265                1270                1275                1280

tat gta aac aac caa cca ccg agt acc aag gtg aat gag agc ttc cgt   3888

Tyr Val Asn Asn Gln Pro Pro Ser Thr Lys Val Asn Glu Ser Phe Arg
                1285                1290                1295

gca ctc aaa tct atc aat ggt aac att aac aca atc ctt tcg att aca   3936

Ala Leu Lys Ser Ile Asn Gly Asn Ile Asn Thr Ile Leu Ser Ile Thr
            1300                1305                1310

tct gat caa tcc aag tca cat gaa gac gac acc aag cca gac cta aac   3984

Ser Asp Gln Ser Lys Ser His Glu Asp Asp Thr Lys Pro Asp Leu Asn
        1315                1320                1325

aat gtt gag atg aag gac acg gcc gaa gaa aca aaa ccg tta aga ggt   4032

Asn Val Glu Met Lys Asp Thr Ala Glu Glu Thr Lys Pro Leu Arg Gly
    1330                1335                1340

ggc gtc gtc gat ctg aat gtg gtg gag gga gag gag aac att gct gaa   4080

Gly Val Val Asp Leu Asn Val Val Glu Gly Glu Glu Asn Ile Ala Glu
1345                1350                1355                1360
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gct agt gga agt gtt gat gta aaa atg gaa gaa gcc aaa gaa gaa gag 4128

Ala Ser Gly Ser Val Asp Val Lys Met Glu Glu Ala Lys Glu Glu Glu
1365 1370 1375

aag cca aag aac atg gtc gtt gat tgactcaact ggtaaatcaa gattc 4177

Lys Pro Lys Asn Met Val Val Asp
1380

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Met Ser Ser Leu Val Glu Arg Leu Arg Ile Arg Ser Asp Arg Lys Pro
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Val Tyr Asn Leu Asp Asp Ser Asp Asp Asp Asp Phe Val Pro Lys Lys
20 25 30

Asp Arg Thr Phe Glu Gln Val Glu Ala Ile Val Arg Thr Asp Ala Lys
35 40 45

Glu Asn Ala Cys Gln Ala Cys Gly Glu Ser Thr Asn Leu Val Ser Cys
50 55 60

Asn Thr Cys Thr Tyr Ala Phe His Ala Lys Cys Leu Val Pro Pro Leu
65 70 75 80

Lys Asp Ala Ser Val Glu Asn Trp Arg Cys Pro Glu Cys Val Ser Pro
85 90 95

Leu Asn Glu Ile Asp Lys Ile Leu Asp Cys Glu Met Arg Pro Thr Lys
100 105 110

Ser Ser Glu Gln Gly Ser Ser Asp Ala Glu Pro Lys Pro Ile Phe Val
115 120 125

Lys Gln Tyr Leu Val Lys Trp Lys Gly Leu Ser Tyr Leu His Cys Ser
130 135 140

Trp Val Pro Glu Lys Glu Phe Gln Lys Ala Tyr Lys Ser Asn His Arg
145 150 155 160

Leu Lys Thr Arg Val Asn Asn Phe His Arg Gln Met Glu Ser Phe Asn
165 170 175

Asn Ser Glu Asp Asp Phe Val Ala Ile Arg Pro Glu Trp Thr Thr Val
180 185 190

Asp Arg Ile Leu Ala Cys Arg Glu Glu Asp Gly Glu Leu Glu Tyr Leu
195 200 205

Val Lys Tyr Lys Glu Leu Ser Tyr Asp Glu Cys Tyr Trp Glu Ser Glu
210 215 220

Ser Asp Ile Ser Thr Phe Gln Asn Glu Ile Gln Arg Phe Lys Asp Val
225 230 235 240

Asn Ser Arg Thr Arg Arg Ser Lys Asp Val Asp His Lys Arg Asn Pro
245 250 255

Arg Asp Phe Gln Gln Phe Asp His Thr Pro Glu Phe Leu Lys Gly Leu
260 265 270

Leu His Pro Tyr Gln Leu Glu Gly Leu Asn Phe Leu Arg Phe Ser Trp
275 280 285

Ser Lys Gln Thr His Val Ile Leu Ala Asp Glu Met Gly Leu Gly Lys
290 295 300

Thr Ile Gln Ser Ile Ala Leu Leu Ala Ser Leu Phe Glu Glu Asn Leu
305 310 315 320

Ile Pro His Leu Val Ile Ala Pro Leu Ser Thr Leu Arg Asn Trp Glu
325 330 335

Arg Glu Phe Ala Thr Trp Ala Pro Gln Met Asn Val Val Met Tyr Phe
340 345 350

Gly Thr Ala Gln Ala Arg Ala Val Ile Arg Glu His Glu Phe Tyr Leu
355 360 365

Ser Lys Asp Gln Lys Lys Ile Lys Lys Lys Lys Ser Gly Gln Ile Ser
370 375 380

Ser Glu Ser Lys Gln Lys Arg Ile Lys Phe Asp Val Leu Leu Thr Ser
385 390 395 400

Tyr Glu Met Ile Asn Leu Asp Ser Ala Val Leu Lys Pro Ile Lys Trp
405 410 415

Glu Cys Met Ile Val Asp Glu Gly His Arg Leu Lys Asn Lys Asp Ser
420 425 430

Lys Leu Phe Ser Ser Leu Thr Gln Tyr Ser Ser Asn His Arg Ile Leu
435 440 445

Leu Thr Gly Thr Pro Leu Gln Asn Asn Leu Asp Glu Leu Phe Met Leu
450 455 460

Met His Phe Leu Asp Ala Gly Lys Phe Gly Ser Leu Glu Glu Phe Gln
465 470 475 480

Glu Glu Phe Lys Asp Ile Asn Gln Glu Glu Gln Ile Ser Arg Leu His
485 490 495

Lys Met Leu Ala Pro His Leu Leu Arg Arg Val Lys Lys Asp Val Met
500 505 510

Lys Asp Met Pro Pro Lys Lys Glu Leu Ile Leu Arg Val Asp Leu Ser
515 520 525

Ser Leu Gln Lys Glu Tyr Tyr Lys Ala Ile Phe Thr Arg Asn Tyr Gln
530 535 540

Val Leu Thr Lys Lys Gly Gly Ala Gln Ile Ser Leu Asn Asn Ile Met
545 550 555 560

Met Glu Leu Arg Lys Val Cys Cys His Pro Tyr Met Leu Glu Gly Val
565 570 575

Glu Pro Val Ile His Asp Ala Asn Glu Ala Phe Lys Gln Leu Leu Glu
580 585 590

Ser Cys Gly Lys Leu Gln Leu Leu Asp Lys Met Met Val Lys Leu Lys
595 600 605

Glu Gln Gly His Arg Val Leu Ile Tyr Thr Gln Phe Gln His Met Leu
610 615 620

Asp Leu Leu Glu Asp Tyr Cys Thr His Lys Lys Trp Gln Tyr Glu Arg
625 630 635 640

Ile Asp Gly Lys Val Gly Gly Ala Glu Arg Gln Ile Arg Ile Asp Arg
645 650 655

Phe Asn Ala Lys Asn Ser Asn Lys Phe Cys Phe Leu Leu Ser Thr Arg
660 665 670

Ala Gly Gly Leu Gly Ile Asn Leu Ala Thr Ala Asp Thr Val Ile Ile
675 680 685

Tyr Asp Ser Asp Trp Asn Pro His Ala Asp Leu Gln Ala Met Ala Arg
690 695 700

Ala His Arg Leu Gly Gln Thr Asn Lys Val Met Ile Tyr Arg Leu Ile
705 710 715 720

Asn Arg Gly Thr Ile Glu Glu Arg Met Met Gln Leu Thr Lys Lys Lys
725 730 735

Met Val Leu Glu His Leu Val Val Gly Lys Leu Lys Thr Gln Asn Ile

740 745 750

Asn Gln Glu Glu Leu Asp Asp Ile Ile Arg Tyr Gly Ser Lys Glu Leu
755 760 765

Phe Ala Ser Glu Asp Asp Glu Ala Gly Lys Ser Gly Lys Ile His Tyr
770 775 780

Asp Asp Ala Ala Ile Asp Lys Leu Leu Asp Arg Asp Leu Val Glu Ala
785 790 795 800

Glu Glu Val Ser Val Asp Asp Glu Glu Glu Asn Gly Phe Leu Lys Ala
805 810 815

Phe Lys Val Ala Asn Phe Glu Tyr Ile Asp Glu Asn Glu Ala Ala Ala
820 825 830

Leu Glu Ala Gln Arg Val Ala Ala Glu Ser Lys Ser Ser Ala Gly Asn
835 840 845

Ser Asp Arg Ala Ser Tyr Trp Glu Glu Leu Leu Lys Asp Lys Phe Glu
850 855 860

Leu His Gln Ala Glu Glu Leu Asn Ala Leu Gly Lys Arg Lys Arg Ser
865 870 875 880

Arg Lys Gln Leu Val Ser Ile Glu Glu Asp Asp Leu Ala Gly Leu Glu
885 890 895

Asp Val Ser Ser Asp Gly Asp Glu Ser Tyr Glu Ala Glu Ser Thr Asp
900 905 910

Gly Glu Ala Ala Gly Gln Gly Val Gln Thr Gly Arg Arg Pro Tyr Arg
915 920 925

Arg Lys Gly Arg Asp Asn Leu Glu Pro Thr Pro Leu Met Glu Gly Glu
930 935 940

Gly Arg Ser Phe Arg Val Leu Gly Phe Asn Gln Ser Gln Arg Ala Ile
945 950 955 960

Phe Val Gln Thr Leu Met Arg Tyr Gly Ala Gly Asn Phe Asp Trp Lys
965 970 975

Glu Phe Val Pro Arg Leu Lys Gln Lys Thr Phe Glu Glu Ile Asn Glu
980 985 990

Tyr Gly Ile Leu Phe Leu Lys His Ile Ala Glu Glu Ile Asp Glu Asn
995 1000 1005

Ser Pro Thr Phe Ser Asp Gly Val Pro Lys Glu Gly Leu Arg Ile
1010 1015 1020

Glu Asp Val Leu Val Arg Ile Ala Leu Leu Ile Leu Val Gln Glu
1025 1030 1035

Lys Val Lys Phe Val Glu Asp His Pro Gly Lys Pro Val Phe Pro
1040 1045 1050

Ser Arg Ile Leu Glu Arg Phe Pro Gly Leu Arg Ser Gly Lys Ile
1055 1060 1065

Trp Lys Glu Glu His Asp Lys Ile Met Ile Arg Ala Val Leu Lys
1070 1075 1080

His Gly Tyr Gly Arg Trp Gln Ala Ile Val Asp Asp Lys Glu Leu
1085 1090 1095

Gly Ile Gln Glu Leu Ile Cys Lys Glu Leu Asn Phe Pro His Ile
1100 1105 1110

Ser Leu Ser Ala Ala Glu Gln Ala Gly Leu Gln Gly Gln Asn Gly
1115 1120 1125

Ser Gly Gly Ser Asn Pro Gly Ala Gln Thr Asn Gln Asn Pro Gly
1130 1135 1140

Ser Val Ile Thr Gly Asn Asn Asn Ala Ser Ala Asp Gly Ala Gln
1145 1150 1155

Val Asn Ser Met Phe Tyr Tyr Arg Asp Met Gln Arg Arg Leu Val
1160 1165 1170

Glu Phe Val Lys Lys Arg Val Leu Leu Leu Glu Lys Ala Met Asn
1175 1180 1185

Tyr Glu Tyr Ala Glu Glu Tyr Tyr Gly Leu Gly Gly Ser Ser Ser
1190 1195 1200

Ile Pro Thr Glu Glu Pro Glu Ala Glu Pro Lys Ile Ala Asp Thr
1205 1210 1215

Val Gly Val Ser Phe Ile Glu Val Asp Asp Glu Met Leu Asp Gly
1220 1225 1230

Leu Pro Lys Thr Asp Pro Ile Thr Ser Glu Glu Ile Met Gly Ala
1235 1240 1245

Ala Val Asp Asn Asn Gln Ala Arg Val Glu Ile Ala Gln His Tyr
1250 1255 1260

Asn Gln Met Cys Lys Leu Leu Asp Glu Asn Ala Arg Glu Ser Val
1265 1270 1275

Gln Ala Tyr Val Asn Asn Gln Pro Pro Ser Thr Lys Val Asn Glu
1280 1285 1290

Ser Phe Arg Ala Leu Lys Ser Ile Asn Gly Asn Ile Asn Thr Ile
1295 1300 1305

Leu Ser Ile Thr Ser Asp Gln Ser Lys Ser His Glu Asp Asp Thr
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Lys Pro Asp Leu Asn Asn Val Glu Met Lys Asp Thr Ala Glu Glu
1325 1330 1335

Thr Lys Pro Leu Arg Gly Gly Val Val Asp Leu Asn Val Val Glu
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Gly Glu Glu Asn Ile Ala Glu Ala Ser Gly Ser Val Asp Val Lys
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Met Glu Glu Ala Lys Glu Glu Glu Lys Pro Lys Asn Met Val Val
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Asp

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<211> 19

<212> DNA

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<221> misc_feature

<222> 18-19

<223> AFLP Primer EcoRI for AFLP Mapping Analysis in Example 1; n may be a, g, c or t

<400> 3

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<210> 4

<211> 19

<212> DNA

<213> Artificial Sequence

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<222> 17-19

<223> AFLP Primer MseI for AFLP Mapping Analysis in Example 1;
n may be a, g, c or t

<400> 4

gatgagtcct gagtaannn 19

<210> 5

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 2;
sequence complementary to nucleotides 1725-1745 of SEQ ID NO:1

<400> 5

tgttgagcca gttattcacg a 21

<210> 6

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 2;
sequence complementary to nucleotides 1934-1914 in SEQ ID NO:1

<400> 6

acctttccat caattcgctc g 21

<210> 7
<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 2

<400> 7

ccgctcgaga accccaatga ccagctcagt 30

<210> 8

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-21

<223> Primers for PCR of Example 2;
sequence complementary to nucleotides 672-652 of
LEC1 cDNA sequence

<400> 8

ccttcttcac ttatactgac c 21

<210> 9

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 2;
nucleotides 65-85 of ROC3 cDNA sequence

<400> 9

aagtctactt cgacatgacc g 21

<210> 10

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 2;
sequence complementary to nucleotides 524-504 of ROC3
cDNA sequence

<400> 10

cttccagagt cagatccaac c 21

<210> 11

<211> 30

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 4;
represent nucleotides 895-924 in SEQ ID NO:1 wherein nucleotide
907 is changed from "a" to "g"

<400> 11

gaaatgggac taggcaggac aattcaaagc 30

<210> 12

<211> 30

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 4;
represent sequence complementary to nucleotides
924-895 in SEQ ID NO:1, with nucleotide 911 changed from "t" to "c".

<400> 12

gctttgaatt gtcctgccta gtcccatttc 30

<210> 13

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-47

<223> Primers for PCR of Example 4

<400> 13

aagccaaaga acatggtcgt tgatctagag gatcctgaag ctcgaaa 47

<210> 14

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-52

<223> Primers for PCR of Example 4

<400> 14

gaatcttgat ttaccagttg agtcattttt gatgaaacag aagctttttg at 52

<210> 15

<211> 21

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 4;
represent sequence complementary to nucleotides 4152-4132 in SEQ ID NO:1

<400> 15

atcaacgacc atgttctttg g 21

<210> 16

<211> 22

<212> DNA

<213> Arabidopsis thaliana

<220>

<223> Primers for PCR of Example 4;
represent nucleotides 4153-4174 in SEQ ID NO:1

<400> 16

tgactcaact ggtaaatcaa ga 22

<210> 17

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 17

ccgctcgagt gagtagtttg gtggagaggc 30

<210> 18

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 18

ccggaattcc atcggaggaa ccttgttcac 30

<210> 19

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 19

cgcggatccc atcggaggaa ccttgttcac 30

<210> 20

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 20

tgctctagat gagtagtttg gtggagaggc 30

<210> 21

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 21

ccgctcgagc cctcacataa gtttgtctgc 30

<210> 22

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 22

ccggaattcg tcttaggaag tccatcaagc 30

<210> 23

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 23

cgcggatccg tcttaggaag tccatcaagc 30

<210> 24

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-30

<223> Primers for PCR of Example 5

<400> 24

tgctctagac cctcacataa gtttgtctgc 30

<210> 25

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-31

<223> Primers for PCR of Example 6

<400> 25

cgcggatcct ttttccactt ctcagtccgg g 31

<210> 26

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-34

<223> Primers for PCR of Example 4

<400> 26

cttcgaactc gagggatccc catggctagc agct 34

<210> 27

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-34

<223> Primers for PCR of Example 4

<400> 27

gctagccatg gggatccctc gagttcgaag gtac 34

<210> 28

<211> 12

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-12

<223> Primers for forming cassette inserted into pCAMBIA3300 in Example 4

<400> 28

ccaggtacct gg 12

<210> 29

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-20

<223> Primers for forming cassette inserted into pCAMBIA3300 in Example 4

<400> 29

aattccaggt acctggcatg 20

<210> 30

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<222> 1-38

<223> Sequence for forming clone of the rat glucocorticoid receptor in Example 4

<400> 30

tctagaggat cctgaagctc gaaaaacaaa gaaaaaaa 38